Microbially managed organic growing media for greenhouse horticulture

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FACULTY OF BIOSCIENCE ENGINEERING

When the wind of change blows

some build walls

others build windmills

(Chinese proverb)

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Notation index

ADF	Acid detergent fiber
ADM	Absolute dry matter (determined at 105°C)
AMF	Arbuscular mycorrhizal fungi
AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
BNF	Biological nitrogen fixation
CEC	Cation exchange capacity
CEN	European committee for standardization
CFU	Colony forming units
COD	Chemical oxygen demand
DO	Dissolved oxygen
DW	Dry weight
EC	Electrical conductivity
FA	Free ammonia
FNA	Free unionized nitrous acid
GB	Grow bag (peat and coconut fiber based organic growing medium)
GM	Growing media
GMA	Growing media additives
GMC	Growing media constituents
GMRS	Growing media reactor system
Ha	Hectare
HATS	High affinity transport system
HRT	Hydraulic retention time
IF	Inorganic fertilizer solution
LATS	Low affinity transport system
MRM	Microbial resource management
NDF	Neutral detergent fiber
NFT	Nutrient film technique
N_{IF}	Inorganic fertilizer nitrogen (ammonium, nitrite and nitrate)
N_{OF}	Organic fertilizer nitrogen (Kjeldahl-N and urea)
NOB	Nitrite oxidizing bacteria
N_{max}	Maximum nitrogen supply
N_{var}	Variable nitrogen supply
OF	Organic fertilizer solution
PLFA	Phospholipid fatty acid analysis
RW	Rockwool (mineral growing medium without plants showing the hairy roots syndrome)
RWS	Rockwool (mineral growing medium with plants showing the hairy roots syndrome)
SCS	Soilless culture system
SOILANIMAL	Organic soil fertilized with animal-derived material, i.e. blood meal
SOILPLANT	Organic soil fertilized with plant-derived material, i.e. malt sprouts
SPAC	Soil-Plant-Atmospheric Continuum
TAN	Total ammonia nitrogen
Tg	Teragram ($1x10^9$ g)

TKN	Total Kjeldahl nitrogen
tpt	Time point
θ_v	Volumetric water content
θ_r	Residual volumetric water content ($m^3\ m^{-3}$)
Θ_s	Saturated volumetric water content ($m^3\ m^{-3}$)

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CHAPTER 1: INTRODUCTION

1. Introduction

Plants are essential for the existence of life on earth. In association with the microorganisms, plants make the earth livable for all other forms of life including mankind (Dixon and Aldous 2014). The provision of food has been imperative to the success of human development. As stated by Jorge Sampaio (United Nations High Representative for the Alliance of Civilizations) horticulture can manage "a lot…to overcome hunger and ensure food security" especially taking into account that the world's population, particularly in developing countries, will reach 9.1 billion by 2050. Plant production has much to bid as urbanization continues nonstop across the world and the Next Green Revolution may rely on microbes (Graber 2014).

Horticulture is the science and art of producing, improving, marketing, and using fruits, vegetables, flowers, and ornamental plants (Dixon and Aldous 2014). Soilless culture systems are very effective production systems in today's horticultural industry (Gruda 2008). Moreover, there is an increased demand from modern societies to supply fresh produce free from pesticides and that are safe for human consumption (Dixon and Aldous 2014). Supermarkets and retailers require such produce to meet critical standards that are set to have pesticide residue levels at barely detectable amounts. As stated by the German retailer REWE: "We have sustainability as corporate principle, meaning we feel responsible for the environment, towards society, and towards their staff". The four pillars of sustainability "Green Products", "Employees", "Energy, Climate and the Environment" and "Social Involvement" build the center of their sustainable acting.

Intensive soilless culture systems require high levels of inorganic fertilizers. As horticultural crops usually have a high value, the relative cost of these fertilizers is of minor importance for the grower potentially leading to a higher risk of land and water pollution (Dixon and Aldous 2014). European farmers and consumers spend an estimated €15.5 billion per year on synthetic fertilizers, with 76% of this value for nitrogen (N), 16% for phosphorus (P), and 8% for potassium (K). High carbon footprint and pollution are associated with the mining processes of P and K and production of synthetic fertilizers. The use of recovered nutrients, such as organic fertilizers is very promising, as they release nutrients slowly and potentially reduce N leaching loss and challenging, as they rely on microbial activity. Phosphorus and nitrogen can be removed and recovered from wastewaters or even from manure through crystallization, yielding struvite (MAP, $MgNH_4PO_4.6H_2O$), a slow releasing fertilizer (De-Bashan and Bashan 2004; El Diwani et al. 2007). However, currently there is little understanding of the influence of these recovered nutrients on plant performance in soilless culture systems.

Soilless culture systems give the possibility to control the water and nutrient supplies and pathogens. These pathogens or deleterious microorganisms are only a small proportion of the aerial and edaphic microbial communities, which surround crop plants. Since the vast majority of the soil and soilless microbial communities are benign, many of these microorganisms must ultimately offer opportunities for promoting crop growth. The management of the microbial community associated with soil and soilless culture systems is a possible sustainable pathway to increased yield and profits. However, currently there is relatively little understanding of soilless microbial communities, the microbial ecology in the bulk zone and the rhizosphere and its impact on plant performance.

Today horticulture and especially greenhouse horticulture and consequently the growing media industry stand at a crossroad. After an uninterrupted period of improvement in food production and

crop yields, threats such climate change, water scarcity, food security, energy shortages, limited natural resources, high pesticide and fertilizer use all lead to a challenging and problematic production environment. There is a demand for an increased food production and wealth creation but in a cleaner, healthier and sustainable environment (Dixon and Aldous 2014). Greenhouse horticulture and consequently also the growing media industry is well placed for a meaningful role in these social changes, because of its environmental and social dimensions (Altmann 2008).

The aim of this thesis will be to develop smarter sustainable solutions for soilless culture systems in a cleaner environment. The challenge will be to gain knowledge about i) the complex physico-chemical and microbial community interactions in soilless culture systems based on organic growing media and ii) the nitrogen conversions in soilless culture systems in combination with organic fertilizers and organic growing media and iii) soilless cultivation systems based on novel and recovered fertilizer. This will result in new expertise concerning soilless microbial community ecology and activity and opportunities for the use of the next generation of fertilizers from recovered nutrients, such as organic fertilizers and struvite that rely partly or totally on microbial activity. Strategies will be developed to minimize too high ammonium/ammonia concentrations in organic growing media in combination with organic fertilizers and novel soilless culture systems. This knowledge about the prokaryotic/eukaryotic interaction is of major importance for horticulture and consequently also for the growing media industry for decades to come.

2. Soilless culture systems

2.1 Introduction

The term "soilless culture" is defined as the cultivation of plants in systems without soil "*in situ*". The methods of growing plants without soil fall into two general categories (Table 1.1). Liquid culture (true hydroponics), where the nutrient solution is recirculated after re-aeration and adjustment of the acidity and nutrient levels, like the nutrient film technique (NFT) and aggregate culture, where the nutrient solution is supplied to plants via an irrigation system through the growing medium, and excess fertigation solution is allowed to drain away or the fertigation solution is recirculated (Baudoin et al. 2013; Olympios 1999). A FAO report of Winsor and Baudoin (1990) gives a generally accepted classification of soilless culture systems (SCS).

The cultivation of plants, vegetables and fruits in glasshouses has a long history (Sonneveld and Voogt 2009a). The origin of soilless culture systems started in the 17th century when, in 1666, Boyle attempted to grow plants in "vials containing nothing but water", and reported that one species (spearmint, *Raphanza aquatic* L.*)* survived for nine months. However, it was not until the 19th century that Justus Liebig (1803-73) and Knop and Sachs (around 1859) initiated the systematic study of plant nutrition (Cooper 1979). The first person to promote the commercial potential of liquid culture embedded in a layer of sand was Gericke (1929). Based on the Greek name hydro (water) and ponos (labor) the term "hydroponics" was proposed by Setchell (Olympios 1999). The first commercial developments of glasshouses focused on production of vegetables, fruits and flowers and occurred mainly in the North-West area of Europe. The production of the crops in glasshouses was mainly connected with the demand of the market for early fruits and vegetables and the production of crops that could not be grown in the cool and wet climate conditions of North-West Europe.

Table 1.1: Overview of the different hydroponic systems according to Olympios (1999)

"TRUE HYDROPONICS" based on nutrient solutions	"HYDROPONICS" based on aggregate systems		
	Natural constituents	Synthetic constituents	Organic constituents
Static solutions	Sand	Foam mats (PUR)	Sawdust
Circulating solutions	Gravel	Oasis	Bark
Nutrient film technique (NFT)	Mineral wool	Hydrogel	Woodchips
Aeroponics	Glass wool		Peat
	Perlite		Fleece
	Vermiculite		Mark
	Pumice		Coco- soil
	Expanded clay		
	Zeolite		
	Volcanic tuff		
	Sepiolite		

2.2 Advantages, constraints and perspectives for soilless culture systems

The main reasons for the switch from soil to soilless culture systems are numerous. Soilless culture systems guarantee flexibility and intensification and provide high crop yield and high-quality products, (Grillas et al. 2001; Morard 1995). The development of soilless culture systems over the last twenty years was primarily driven by economics, however, there was also an important ecological advantage due to the recirculation of drain water as an important step towards a more environmental friendly vegetable cultivation system. Soilless culture systems are less susceptible to soil-borne diseases and give complete control over water and nutrient supplies (Gruda 2008) compared to soil culture systems. They include alternatives to soil disinfection, soil tillage and preparation, thereby increasing crop productivity and yield (Baudoin et al. 2013). However, these systems require high technical skills (Baudoin et al. 2013), investments and they have to deal with high risk of infections with pathogens, especially in closed soilless culture systems (Rosberg 2014).

The risks associated with biocide residues in soilless culture systems are large. Residues of biocides can accumulate in discharged water from soilless culture systems and may contaminate sewage, soil and surface waters (Wainwright et al. 2014). The worldwide active substance use in vegetable glasshouse horticulture was 8 kg ha^{-1} y^{-1} in 2010. A survey executed by the "Deutsche Fruchthandelsverband" (DFHV) and the "QS Qualität und Sicherheit GmbH (QS)" of 26065 samples coming from 75 different countries showed that no residues were found in 43.1 % of all the analyzed samples. The maximum

residue levels of plant protection products was exceeded in 2.2% of the analyzed samples (Verbaas et al. 2016).

A central environmental impact on water quality and human health is the occurrence of excessive nitrate levels. Nitrogen fertilizers that leach into the ground, and runoff into water courses contaminate surface water. Excessive nitrate (NO_3^-) concentrations in drinking water is associated with methemoglobinemia or "blue-baby" syndrome, an illness of infants under 6 months' old (Knobeloch et al. 2000).

Every year about 450 m^3 ha^{-1} y^{-1} of drainage water or 133 kg N ha^{-1} y^{-1} coming from glasshouses is discharged into the environment during the cultivation of cucumber in soilless culture systems (Beerling et al. 2016). Water quality norms of water leaving the glasshouse with respect to biocides and nutrients have often been exceeded in glasshouse regions, causing a considerable negative impact on the quality of ground and surface waters.

Recent studies have shown that organic farming systems have a better balanced sustainability metrics in regards to performance than their conventional counterparts (Figure 1.1) (Reganold and Wachter 2016).

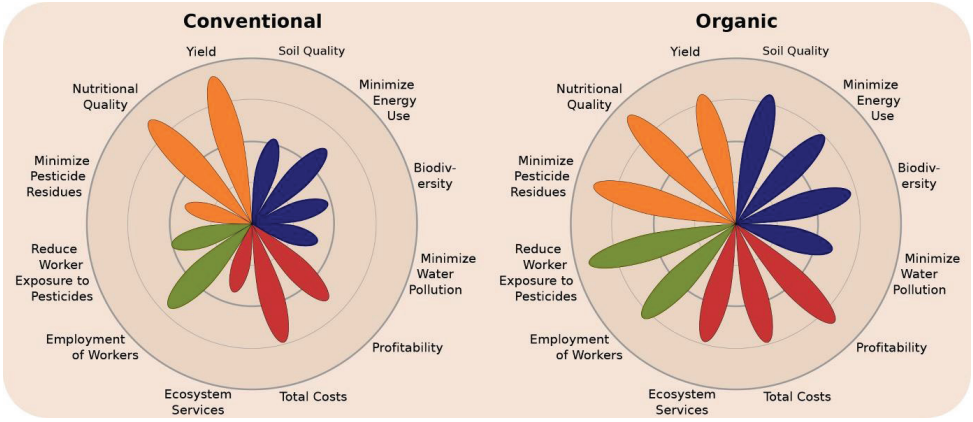


Figure 1.1: Assessment of organic farming relative to conventional farming in the four major areas of sustainability. Lengths of the 12 flower petals are qualitatively based on the studies discussed in the review of Reganold and Wachter (2016) and indicate the level of performance of specific sustainability metrics relative to the four circles representing 25, 50, 75 and 100%. Orange petals represent areas of production; blue petals represent areas of environmental sustainability; red petals represent areas of economic sustainability; green petals represent areas of wellbeing. The lengths of the petals illustrate that organic farming systems better balance the four areas of sustainability (Reganold and Wachter 2016).

In addition, organically produced foods have significantly less to no synthetic pesticide residues compared with conventionally produced foods (Baker et al. 2002). Organic agriculture is designed to improve the biological diversity, to increase soil biological activity, to maintain long-term soil fertility, to recycle wastes of plant and animal, restore soil nutrients, thus minimizing the nonrenewable resources, to promote the healthy use of soil, water and air and minimize all forms of pollution, which

results from agricultural activities. Organic cultivation systems, however, have to deal with lower yields compared with soilless culture systems (Connor and Mínguez 2012; Kirchmann and Thorvaldsson 2000). This knowledge and opportunities stimulated research to develop organic like soilless culture systems, thereby i) using organic growing media that can be composted at the end of the season (Grunert et al. 2008), ii) using organic fertilizer that rely on microbial activity associated with the growing medium and iii) supplying the correct amounts of nutrients at the correct time in relation with the growth of a crop and attaining high yields.

In soilless culture systems, fertilization and irrigation are integrated into one system able to supply fertilizers and water (fertigation), where all the macro- and micronutrients essential for crops can be supplied through water-soluble fertilizing salts (Figure 1.2). The osmotic potential of nutrient solutions is mostly measured by the electrical conductivity (EC) and is build up in nutrient solutions by mineral salts. In general, there is a linear relationship between the osmotic potential and the EC (Sonneveld and Voogt 2009b). As the electrical conductivity is highly dynamic in combination with organic fertilizers due to the mineralization, the electrical conductivity of a fertigation solution in combination with organic fertilizers is not a reliable parameter. Consequently, standard key control parameters such as pH and EC cannot be used and little is known about potential key control parameters in combination with organic fertilizers to supply the correct amount of nutrients to the plants.

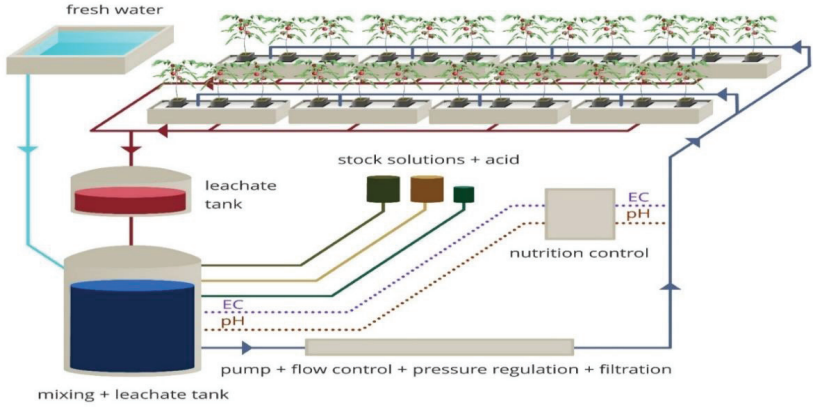


Figure 1.2: Closed-loop soilless culture system used for the cultivation of tomatoes and adapted after Putra and Yuliando (2015). Redrafted by Tim Lacoere.

2.3 Soilless culture systems and growing media: importance and utilization

A growing medium (GM) is often referred to as "substrate", "substratum" or "potting soil". According to the European Committee for Standardization definition, a "growing medium is other material than soil *in situ"*, and can be described as a "containerized" substance through which plant roots grow and extract water and nutrients limited in space and time (CEN 1999). A growing medium is a mixture of one or more growing medium constituents (GMC), which are formulated on a percentage volume basis (% v/v) (Schmilewski 2008) (Figure 1.3). In this PhD thesis also an organic soil (chapter 6) is used, meaning that the soil is certified (PCG, Kruishoutem) and managed organically according to the EU organic farming legislation (EC No 889/2008).

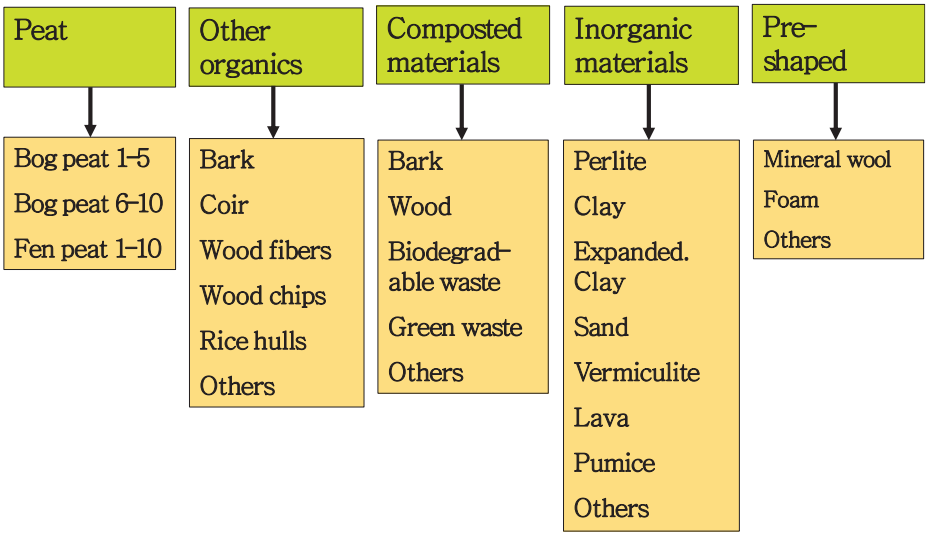


Figure 1.3: Overview of the growing media constituents used in growing media (Schmilewski 2007)

GMC (Picture 1.1 and Figure 1.3) can be divided into 5 different groups: i) peat based materials (weakly decomposed (H1-H5 on the von Post scale), moderately to rather highly decomposed peats (H6-H10 on the von Post scale) and fen peat H1-H10)) (Von Post 1926), ii) other organics (bark, coconut coir, coconut chips, coconut fiber, wood fibers, woodchips, rice hulls), iii) composted materials (bark, wood, biodegradable waste, green waste), iv) inorganic materials (perlite, clay, expanded clay, sand, vermiculite, lava, pumice) and v) pre-shaped materials (mineral wool, foam) (Schmilewski 2008).

Joosten and Clarke (2002) defined peat as sedentarily accumulated material consisting of at least 30% (dry mass) of dead organic material. For better understanding, it is important to add that peat accumulation occurs in mires only. These mires are peatlands, where peat is currently being formed. Peatlands are areas with or without vegetation with a naturally formed peat layer of 30 cm or more on the surface. This definition for peatland is valid in most EU countries and other countries worldwide (Altmann 2008).

Raised bogs are the most commonly used peatland types for peat extraction. Bogs are described as ombrotrophic, due to their very low nutrient status. Bog water has a low pH value and oxygen content which inhibits the decomposition of organic matter. As a result, only plant species well adapted to this habitat can grow in bogs. Peat moss, *Sphagnum* spp., dominates bog vegetation and accounts for most of the peat that accumulates in bogs. Other species common to bogs are cotton grass (*Eriophorum* spp.), pink bog rosemary (*Andromeda polifolia*), heath (*Erica tetralix*), heather (*Calluna vulgaris*) and others. The degree of decomposition (or humification) of peat is decisive for its quality, extraction method and usage. The H1 to H10 Von Post humification scale (Von Post 1926) describes the degree of peat decomposition and is commonly used worldwide. The higher the H-rating the more decomposed or humified the peat. Peat can have different meanings: the undisturbed peat on the original site of the peat bogs and the harvested or processed and piled peat. In this thesis, peat is used in the sense of processed material and 'white' peat is extracted mainly by surface milling (Irish peat) or sod cutting (sod peat).

Growing media additives (GMA) are additional ingredients of mixes, which are usually added to the blend on a weight basis. Additives include fertilizers (inorganic and organic fertilizer according to National Belgian legislation, KB 28 of January 201), lime, buffering materials, binders, wetting agents, hydrogels, chemical pesticides, microorganisms and substances, dyes and other substances (Schmilewski 2008). According to the Belgian legislation (National Belgian legislation, KB 28th of January 2013) an organic fertilizer is a fertilizer that contains only plant and/or animal derived organic substances.

The first growing media were mainly composed of soil *ex situ* followed by mixtures of different soils, soils with organic matter, and, starting from the 1950s until the 1970s, media in which soil was replaced by a growing medium constituent and finally pure peat-based growing media were used (Aaron 1982; Hoitink and Poole 1980; Solbraa 1979). Peat is an important constituent in growing media and indispensable to vegetable and mushroom growing, floriculture and nursery management (Jackson 2008), due to its excellent chemical, physical and biological properties (Schmilewski 2008).

The main function of a growing medium is to physically support plants and to supply adequate oxygen, water and nutrients to ensure optimal plant functions (Handreck and Black 2002; Jackson 2008; Lemaire 1994; Reinikainen 1992). An optimized growing medium is just as important as water and fertilizers, and is used in large variety of horticultural applications, from sowing bedding plants or herbs to planting balcony plants and shrubs and trees.

Picture 1.1: (left) source European Peat and Growing Media Association (EPAGMA) and (right) Substrate e.V (eingeschriebener Verein)

In 2007 (Altmann 2008), 37 million m³ of growing media were produced in the EU countries; over 22 million m³ of this was for the professional market and about 15 million m³ for the hobby sector. Peat usage accounted for a volume of 29 million m³. Peat-free growing media occupy a subordinate position in growing media production in the EU (Altmann 2008), however it is getting more and more important. Nowadays the peat and growing media industry represents a €1.3 billion turnover accounting for 11,000 jobs across Europe and is essential for the horticulture industry, which is estimated to have a turnover of approximately €60 billion and provides over 750,000 jobs (Altmann 2008).

2.4 Growing media in relation to vegetable production

Tomatoes are the most produced fruit vegetables in the world. The annual average worldwide production of tomatoes has been estimated at 3.48 kg m^{-2} (FAOSTAT 2013). The worldwide production of vegetables and tomatoes has increased since 2000 with 44% for vegetables and 49% for tomatoes,

which indicates the economic importance of this crop. The total production of tomatoes among the EU-28 Member States was 15,672 million tonnes in 2014 and with a production area of 247,880 ha (Eurostat 2014). In Belgium, the total tomato production area in 2015 was 491 ha. Tomatoes in Belgium are mainly produced for the fresh market, using cultivars with indeterminate growth and attaining almost year-round production of up to 35 trusses per plant in modern glasshouses. This practice allows yields of up to 60 kg m^{-2} or even higher.

In the U.S.A, Canada and Europe, 95% of glasshouse vegetables, and especially tomatoes, are produced in soilless culture systems (Peet and Welles 2005). In Belgium 97% of the tomatoes were cultivated with soilless culture systems, with an average yield of 48.9 kg m^{-2} (Anonymous 2015). In The Netherlands and Belgium, nearly all vegetables like tomatoes, egg plants, cucumbers and peppers, grown in glasshouses are produced on mineral growing media (Islam 2008; Sonneveld 1989).

For the cultivation of tomatoes (Picture 1.2) about 10-12 L of growing medium per m^{-2} of soil surface is recommended (Heuvelink 2005), however practice uses about 7-7.5 L growing medium per m^{-2} of soil surface. Volumes of 5 and 16 L growing medium per m^{-2} of soil surface can also be used, but higher volumes increase the cost of the growing medium per square meter and lower volumes leave little buffer for errors in fertigation (Heuvelink 2005). Mineral growing media have good aeration and water holding capacity and provide a complete control over nutrient and water supply and they are almost biologically inert, making them initially free of any pests, diseases and any kind of weeds. Mineral growing media are manufactured by heating basaltic rock until 1500°C (Pluimers 2001), and are usually provided as plastic wrapped slabs of spun wool (Heuvelink 2005).

Picture 1.2: Growing media used for the cultivation of tomatoes in a soilless culture system (O. Grunert, 2016) Picture taken at Kruishoutem, Proefstation voor de groenteteelt in 2016

Growing media based on peat and peat with coconut fiber (Picture 1.2) showed that tomato plants grown in the pure peat rooted more easily than those grown in the peat-coconut fiber or mineral growing media but the total yield was not significantly different (Grunert et al. 2008). Similar yields of 15-16 kg m^{-2} after 34 weeks of cultivation were obtained with tomato plants grown in straw and

mineral growing (Nurzyński 2006). Tomato plants grown in perlite produced higher total marketable yield than plants grown in pine bark or mineral growing media (Hanna 2009). Some studies have shown that vegetables grown in organic growing media are tastier than those grown in inorganic media (Grunert et al. 2008; Luoto 1984). Several researchers tested organic fertilizers in combination with peat, perlite and compost and made important contributions towards the establishment of sustainable horticulture (Brentlinger 2005; Gruda 2008; Liedl et al. 2004; Peet et al. 2004; Succop and Newman 2004). Based on the results of the mentioned studies, organic growing media are promising and competitive alternatives to mineral growing media.

2.5 Growing medium in relation to the plant and the atmosphere

Many processes in the growing medium-plant-water-continuum are influenced by the amount of water, gas exchange with the atmosphere, diffusion of air and nutrients to the plant roots, temperature of the growing medium, the activity and ecology of the microbial community associated with the growing medium, and the rate at which dissolved chemicals are transported to the root zone (Cornelis et al. 2009). Equation 1 (Cornelis et al. 2009) shows that the water potential (ψ) is composed of four components, representing the different forces that can act on water molecules:

$$\Psi = \psi_p + \psi_\pi + \psi_m + \psi_g \qquad \text{Eq. 1}$$

where Ψ= water potential

ψ_p= hydrostatic potential

ψ_o= osmotic potential

ψ_m= matric potential

ψ_g= gravitational potential

Total soil or growing medium water potential is the sum of the hydrostatic, osmotic, matric and gravitational potential (Eq.1). The gravitational potential is constant, whereas matric and osmotic potentials can change considerably. The hydrostatic pressure refers to the physical pressure exerted on water in the system. In non-saline soils and growing media, matric potential is the dominant component. As the matric potential decreases, the water availability decreases because the water is held more tightly to the aggregate surfaces (Ilstedt et al. 2000). Consequently, nutrient diffusion is restricted and microorganisms may become nutrient-limited (Chowdhury et al. 2011b). Osmotic potential is a function of matric potential, because osmotic potential decreases with decreasing matric potential due to the increasing salt concentration. Hence, microorganisms will also experience decreases in osmotic potential as the soil or growing medium dries. Decreasing matric potential decreases carbon and nitrogen mineralization rate (Pulleman and Tietema 1999), cell numbers, fungal abundance and the capacity of soil and soilless microbial communities to decompose carbon sources (Chowdhury et al. 2011b).

The effect of decreasing matric potential on the bacteria/fungi ratio varies and there are some contradictory reports. Some fungi can withstand low water potential (Harris 1981) and drought can result in a relative increase in fungal biomass (Chowdhury et al. 2011b). However, a decrease in fungal

biomass or an increase in the bacteria/fungi ratio with decreasing soil water content has also been reported (Frey et al. 1999; Stromberger et al. 2007).

The relation between the volumetric water content (θ_v) and the matric potential (h) of a certain growing medium can be expressed by the water retention curve. Hydraulic characterization of growing media is often conducted by fitting measurement data to the van Genuchten (1980) equation (Eq.2):

$$\theta(h) = \theta_r + \frac{\theta s - \theta r}{((1+|\alpha h|^n)^{(1-\frac{1}{n})}} \qquad \text{Eq. 2}$$

θ_r and θ_s are the residual and saturated volumetric water content ($m^3\ m^{-3}$), respectively, and α (cm^{-1}) and n (dimensionless) the form parameters (De Swaef 2011). Θ (h) is the volumetric water content in function of the matric potential. The volumetric water content (θ_v) of a mineral growing medium is very high near saturation (approximately 0.95 $m^3\ m^{-3}$), but decreases substantially with decreasing matric potential (h), with θ_v approaching zero at -5 kPa (De Swaef et al. 2012). As a result of this low water buffering capacity, hydraulic conductivity (K), which is a measure of the ability a soil or growing medium to transmit water, decreases dramatically with decreasing matric potential (h). Such a decrease in the hydraulic conductivity (K) might cause water to be less available for plant roots, even at slightly negative h-values. Growing media with a coarser structure have in generally higher saturated hydraulic conductivities (Ks) values than media with a finer structure (Cornelis et al. 2009).

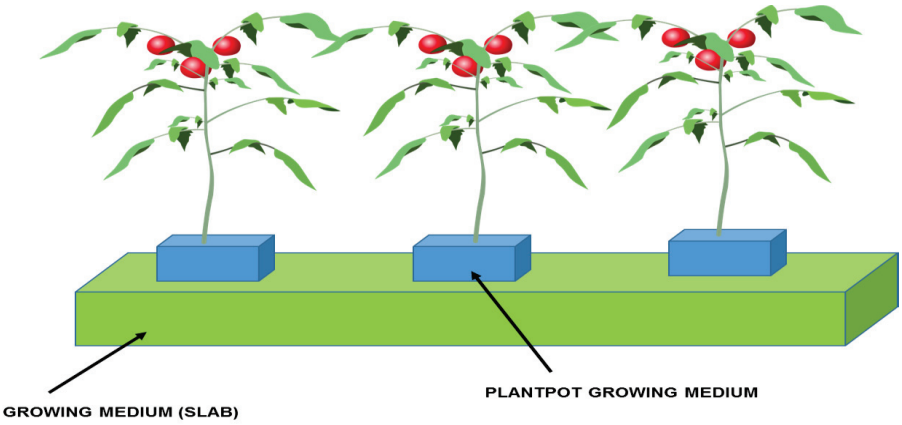


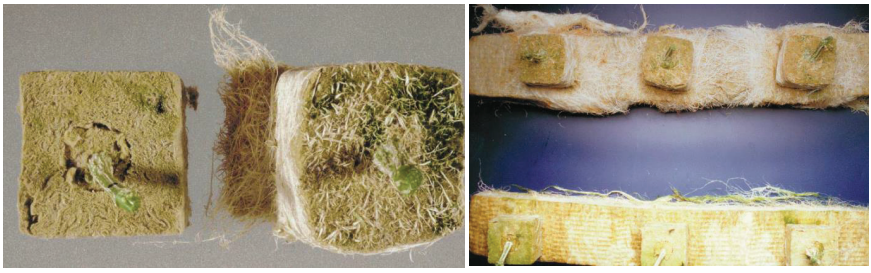
Figure 1.4: Overview of a typical setup for the cultivation of tomatoes in soilless culture systems with growing media

In addition, for the cultivation of tomatoes, several kind of slabs filled with different GMC (Figure 1.4) or even blends of the different constituents are used. On top of these slabs, a plant pot-growing medium is placed, also made of one or more different growing media constituents (Figure 1.4). Consequently, it can be expected that this combination of different growing media will finally impact the water potential and plant water potential (Cornelis et al. 2009) and the flow of water in the growing medium-plant-water-continuum.

2.6 New microbial threats in soilless culture systems – the hairy roots syndrome

The productivity of soilless culture systems relies heavily on increased yields. Since the last decade, the 'hairy root disease' or 'crazy roots' or "root mat syndrome" caused by the pathogen *Agrobacterium rhizogenes* or rhizogenic *Agrobacterium* has become a major problem in soilless culture systems (Picture 1.3). This hairy root disease is characterized by extensive root proliferation and occurs in many economically important crops such as tomatoes (Bosmans et al. 2015a) and egg plants (Grunert et al. 2016a). It was first described as a soil borne disease of economic importance on apples in the early 20th century (Hildebrand 1934). However, since the early 1990s, this was also detected in soilless culture systems with cucumber plants and tomato crops (Weller et al. 2000), resulting in significant yield losses of up 5-10% and consequently also economic losses.

These pathogenic strains are very well known for their formation of biofilms. Biofilms are difficult to eliminate as they provide a niche where the pathogens are protected against disinfectants (Bosmans et al. 2015b). This makes these pathogens an important risk factor in open and even more in closed soilless culture systems where the irrigation water is circulated.



Picture 1.3: (Left) Propagation cube made of mineral growing media (right) swollen by root proliferation caused by cucumber hairy roots disease, compared with a healthy cube (left). (Right) Mineral growing media (top) on which infected plants had been propagated, showing extensive root proliferation across the slab surface compared with a healthy control slab (bottom) (Weller et al. 2000)

Disinfection treatments that are commonly applied in soilless culture systems include chlorine-based disinfectants and hydrogen peroxide (H_2O_2). It was demonstrated that the use of H_2O_2 are effective to reduce biofilm formation by rhizogenic agrobacteria. Nevertheless, it was also clear from the results obtained by Bosmans et al. (2016b) that the required H_2O_2 concentrations depend on the particular *Agrobacterium* strains present in the greenhouse. For the catalase positive *Agrobacterium* population, a treatment of 100 ppm H_2O_2 was required to be effective compared to a catalase negative *Agrobacterium* population a treatment with 50 ppm H_2O_2 was sufficient. The use of these disinfection measures often results in the elimination of not only deleterious microorganisms, but also of potentially beneficial microorganisms for the plant. This may ultimately prevent the soilless culture community from reaching equilibrium and stability, making these soilless culture systems at risk of successful pathogen invasion, such as the hairy roots syndrome caused by *Agrobacterium rhizogenes*.

Mineral growing media start with a 'microbiological vacuum', lacking a diverse and competitive microbial community (Postma 2009). Within these communities, bacteria compete with their neighbors for space and resources. Competition among microorganisms for resources in a habitat depends on several factors, including rates of nutrient uptake, inherent metabolic rates, and

ultimately, growth rates. Garbeva et al. (2004) hypothesized that in a stable system, each microhabitat is occupied by organisms capable of colonizing niches. A diverse and stable ecosystem at the microhabitat level will resist environmental stresses and potentially, pathogen invasion. Mendes et al. (2011) suggested that the relative abundance of several bacterial taxa may be an indicator of disease suppression. In this way, increased resistance to pathogen invasion may be related to the total microbial biomass in the growing medium, which competes with pathogens for resources or may cause inhibition through direct antagonism.

The use of biocontrol organisms seems to be very promising, because they are not harmful to non-target organisms and less damaging to the environment than chemical pesticides (Bosmans et al. 2016b). As indicated by Bosmans et al. (2016a), the nutrient composition of the agar affects *in vitro* screening of biocontrol activity of antagonistic microorganisms. Hence, there is a clear indication that the physico-chemical environment is an important factor whether biocontrol organisms can inhibit the pathogen. However, a large knowledge gap exists concerning the most significant physico-chemical factors in relation to the microbial community composition in soilless culture systems, especially in mineral and organic growing media and the presence of pathogens.

3. Nitrogen

3.1 The terrestrial nitrogen cycle

Nitrogen (N) is an essential element of many compounds found in living cells of microorganisms, plants, animals and humans (Figure 1.5). The element nitrogen is extremely abundant, making up 78% (v/v) of the earth's atmosphere; however, it exists mainly as unreactive di-nitrogen (N_2). Different natural and anthropogenic processes are known to deposit reactive forms of nitrogen on earth accounting for 413 Tg N y^{-1}. The natural biological nitrogen fixation (BNF) by marine and terrestrial ecosystems accounts for 140 Tg N y^{-1} and 58 Tg N y^{-1} respectively and N fixed by lightning accounts for 5 Tg N y^{-1} (Fowler et al. 2013).

The anthropogenic N deposition is facilitated by the Haber–Bosch process, where H_2 and N_2 are combined at high temperatures (300-350°C) and pressures (15-25 MPa) in the presence of catalyst (Chagas 2007) and accounts for 210 Tg N y^{-1}. About 80% of the Haber-Bosch N is used for the production of agricultural fertilizers resulting in the fact that enough food can be produced to feed about 6 billion people (Erisman et al. 2008).

Nitrogen fixing bacteria convert N_2 into ammonia, which is usually quickly assimilated into proteins and other organic nitrogen compounds carrying NH_2 groups. These organic nitrogen compounds are subsequently transformed into ammonia (NH_3) and a carbon component by a process called mineralization or ammonification. Once in the form of ammonia, nitrogen can be further converted to nitrate (NO_3^-) through the process called nitrification. The oxidized forms of nitrogen such as nitrite and nitrate can be transformed back to dinitrogen gas by the process of denitrification. The dissimilatory nitrate reduction to ammonium (DNRA) and the anammox process constitutes a short circuit in the N cycle as it converts ammonia together with nitrite into dinitrogen gas (Strous et al. 1999; Strous et al. 1998). The following three processes (nitrogen fixation, anammox and DNRA), will not be further elaborated here, as they are beyond the scope of this work.

The massive input of Haber-Bosch nitrogen on Earth however resulted in a disturbance of the natural system capable of effectively conserving and re-using reactive nitrogen compounds. Consequently, a more sustainable nutrient management by using for instance recovered nutrients to ensure economic growth, food security and move towards more sustainable production systems is needed. Organic fertilizers can be considered as a blend of recovered nutrients and can be produced on-farm such as slurries, poultry manures, digestate or off farm coming from food industry residues (Hajdu et al. 2015). In addition, nitrogen can be removed and recovered from wastewaters or even from manure through struvite crystallization, yielding struvite (MAP, $MgNH_4PO_4.6H_2O$), which is a promising slow releasing fertilizer (De-Bashan and Bashan 2004; El Diwani et al. 2007).

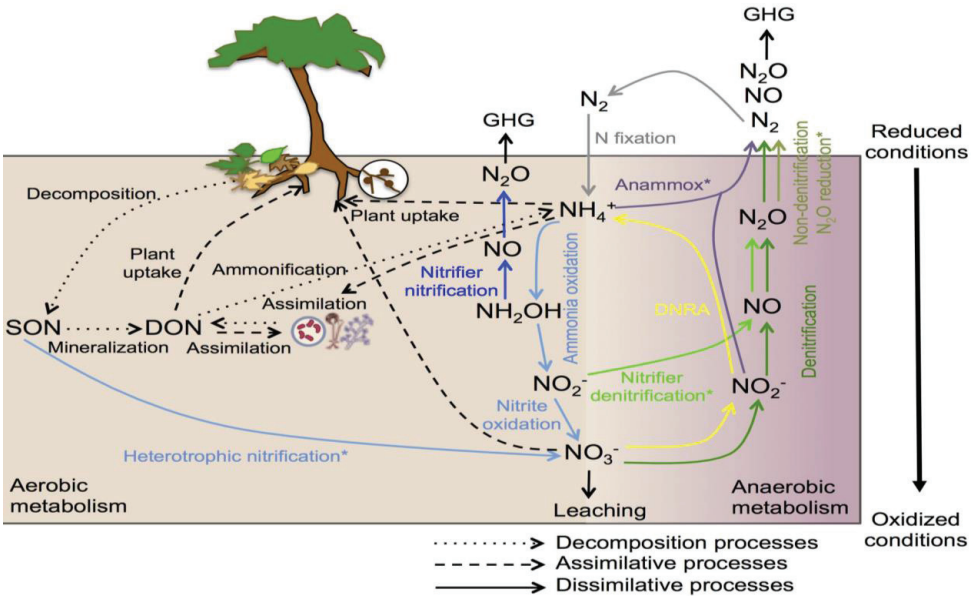


Figure 1.5: Schematic representation of the biological N cycle in soil. The arrows of decomposition, assimilative and abiotic processes are shown in black and the arrows of dissimilative processes are shown in different colors: N-fixation (gray), nitrification (light blue), nitrifier nitrification (dark blue), nitrifier denitrification (light green), denitrification (dark green), non-denitrification N_2O reduction (olive green), DNRA (yellow), and anammox (purple). DNRA, dissimilatory nitrate reduction to ammonium; SON, soil organic nitrogen; DON, dissolved organic nitrogen; GHG, greenhouse gas. Redrafted after Pajares and Bohannan (2016)

3.2 Nitrogen in relation to plants

3.2.1 Introduction

Nitrogen is a major nutrient required in the highest amount of all macronutrients. About 1-5% of the dry matter of a plant consists of nitrogen. Nitrogen is an important constituent of proteins and nucleic acids, being the two most important classes of macromolecules next to chlorophyll, co-enzymes,

phytohormones and secondary metabolites (Marschner 2011). The availability of nitrogen by the plant roots has an important impact on the growth of the plants. Plants can use a wide array of chemical N forms, ranging from simple inorganic N compounds such as ammonium (NH_4^+), nitrate (NO_3^-) and to polymeric N forms such as proteins (Paungfoo-Lonhienne et al. 2008). The major nitrogen forms taken up by the plant are ammonium and nitrate (Marschner 2011).

3.2.2 Uptake of organic nitrogen by plants

Amino acid N is in many cases used as a synonym and a model compound, especially glycine, for organic nitrogen uptake by the plants (Näsholm et al. 2009). Free amino acids only account for a small fraction of the organic nitrogen pool (Näsholm et al. 2009) in the soil. Peptide- and protein-bound amino acids contribute to more than half of the organic nitrogen pool (Senwo and Tabatabai 1998). These polymeric nitrogen forms are the sources for the production of the monomeric forms. In soilless culture systems, the organic derived nitrogen originates mainly from the use of organic fertilizers that are blended with the growing medium. The combined organic fertilizer is a blend of different materials, such as plant-derived material like soya, cacao or animal-derived material like blood meal and feather meal, but also from microbial biomass (Coppens et al. 2015) with a well known and constant nutrient content. Important characteristics of the organic fertilizers are the N-P-K content of the dry matter, the organic matter content and the C/N ratio (Vandenberge et al. 2010).

Plants have a high capacity to take up and metabolize L-α-amino acids. Fungi, bacteria and animals, on the contrary, have the capacity to metabolize D-amino acids. Although D-Alanine (Ogawa et al. 1978) and D- tryptophan (Gamburg and Rekoslavskaya 1991) are also metabolized, this ability is not well developed in plants (Näsholm et al. 2009; Valle and Virtanen 1965), resulting in accumulation of D-amino acids in plants (Brückner and Westhauser 2003). The low capacity to metabolize compounds such as D-Alanine and D-Serine results in toxic effects on plants (Erikson et al. 2004; 2005). Amino acids are absorbed through proton symport with either one or two protons transported simultaneously with the amino acid (Bush 1993). Some amino acids, such as L-Glutamine, can also affect root development (Walch-Liu et al. 2006). Micromolar concentrations of L-Glutamate are perceived specifically at the primary root tip and inhibit mitotic activity in the root apical meristem, but does not interfere with lateral root initiation or outgrowth.

3.2.3 Uptake of ammonium by the plants

3.2.3.1 Ammonium uptake is facilitated through two different transport systems

Ammonium (NH_4^+) is in equilibrium with free ammonia (NH_3), which is a weak base with a pK_a value of 9.25. Growing media, generally, have a lower pH(H_2O) than 9.25, meaning that the actual ammonia (NH_3) concentration is low in growing media. Consequently ammonium is, compared to ammonia, the main nitrogen form present in growing media and taken up by the roots (Loqué and von Wirén 2004).

In sugarcane stems, Tejera et al. (2004) described the existence of ammonium in the apoplastic and symplastic saps. Ammonium uptake (Figure 1.6) by the roots is facilitated through two different transport systems, a high affinity transport system (HATS), that operates at ammonium concentrations lower than 7 mg NH_4^+-N L^{-1} and a low affinity transport system (LATS) that operates at ammonium concentrations higher than 7 mg NH_4^+-N L^{-1}.

The LATS systems is responsible for the ammonium uptake at high concentrations resulting in ammonium toxicity (Marschner 2011). Ammonium transport is carried out by the ammonium transport family (AMT/MEP/Rh) (von Wirén and Merrick 2004). The AMT1 family transports the ammonium via ammonium (NH_4^+) uniport or NH_3/H^+ symport. The *AtAMT1;1* and *AtAMT1;3* are expressed in the root cortical and epidermal cells and directly linked to the ammonium uptake from the growing medium. The *AtAMT1;3* is expressed in the cortical and endodermal root cells, suggesting that AtAMT1.2 is involved in the uptake of ammonium from the apoplast for radial transport of ammonium (Figure 1.6) (Marschner 2011).

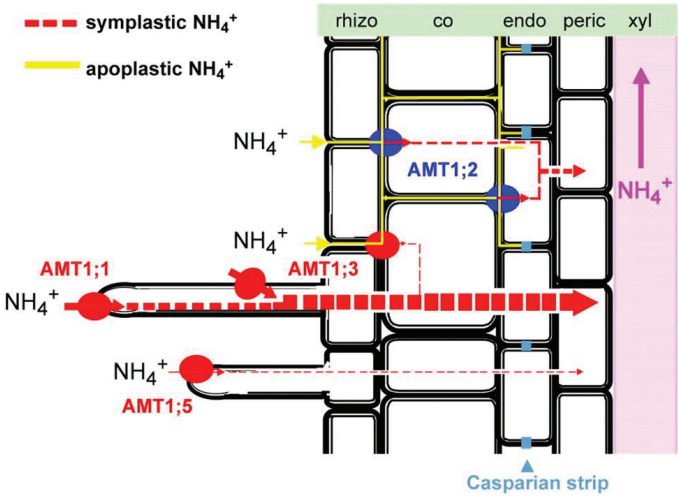


Figure 1.6: Schematic representation of the functions of the AMT1 transporters in the ammonium HATS in Arabidopsis roots. Rhizo: rhizodermis; co= cortex; endo=endodermis; peric = pericycyle; xyl= xylem. Redrafted after Yuan et al. (2007)

3.2.3.2 Sensitivity of plants to ammonium

Sensitivity to ammonium seems to be an universal phenomenon as it has been observed in animals, humans and plants (Britto and Kronzucker 2002). The toxic threshold ammonium concentration varies widely between species. External ammonium concentrations ranging from 1.4 mg NH_4^+-N L^{-1} to 7 mg NH_4^+-N L^{-1} may lead to toxicity symptoms in plants (Britto and Kronzucker 2002). The symptoms of ammonium toxicity include leaf chlorosis, stunted growth, necrotic leaves, inhibition of the primary root growth, and, in severe cases even plant death (Marschner 2011; Qin et al. 2011). Several theories have been put forward to explain ammonium toxicity in plants, like the acidification of external growth environment, displacement of important cations, such as potassium and magnesium, or excessive energy being wasted due to the toxic ammonium (NH_4^+) requiring removal from the cells (Britto and Kronzucker 2002; Qin et al. 2011).

There are several anaplerotic reactions, *i.e.* chemical reactions that form intermediates of a metabolic pathway, that can provide the necessary carbon for this formation of C-N bonds (Ariz et al. 2013). Ammonium conjugation to glutamic acid to form glutamine and the synthesis of glutamic acid from 2-oxoglutaric acid are considered as the critical pathways of ammonia assimilation and to protect cells from ammonium toxicity and is a feedback loop on N_2 fixation. Ammonium can also be produced under

environmental stress (Bittsánszky et al. 2015). To achieve a high ammonium tolerance strategies such as the gradual increase of the ammonium concentration instead of the currently applied shock loads have high potential (van der Ha 2013).

3.2.3.3 Ammonium uptake counteracts potassium, calcium and magnesium uptake

Ammonium resembles potassium in terms of ionic size and size of the hydration shell, thus, ammonium ions are able to pass through potassium-channels. The low concentration of potassium (ten Hoopen et al. 2010) in ammonium fed plants leads to an upregulation of the potassium-channels to boost the potassium uptake, potentially resulting even in a higher ammonium influx though these channels. Next to low potassium concentrations, also the content of other essential cations, like calcium and magnesium in the plant are decreased and increased levels of chloride, sulphate and phosphate were found (Britto and Kronzucker 2002).

3.2.4 Nitrate uptake by the plant roots

Although it is common knowledge that plants assimilate nitrate, less well known is that nitrite can also serve as a nitrogen source for many plants, provided it is available in appropriate concentrations (Aslam et al. 1992). Similar to ammonium, nitrate is taken up from the growing media by the plant by two different nitrate uptake systems, with NRT1 the high-affinity transport system (HATS), and NRT2 the low-affinity transport system (LATS). Tejera et al. (2006) reported the presence of nitrite and nitrate in the apoplastic and symplastic sap of field grown sugarcane cultivated with high N fertilization. The energy needed for the uptake of nitrate is provided by proton gradients (Crawford and Glass 1998; Meharg and Blatt 1995; Wang and Crawford 1996). Uptake of nitrate from the rhizosphere into the symplast takes place against an electrochemical potential gradient (Lambers and Chapin III 2000), which depends on respiratory energy and takes place against an ascending concentration gradient. The nitrate transporters NRT1 and NRT2 transport nitrate across the plasma membrane in symport with protons (Forde 2000), and in turn requires ATP by the H^+-ATPase for proton extrusion to maintain the proton gradient over the plasma membrane (Lambers and Chapin III 2000). Nitrate as such is non-toxic (Mensinga et al. 2003; Speijers and Van Den Brandt 1996).

3.2.5 Influence of nitrogen form on pH dynamics in the rhizosphere

The rhizosphere can be defined as the volume of soil surrounding the roots, which is influenced by root activity (Hiltner 1904). The most important factor influencing the root-induced changes in the rhizosphere is the uptake of nutrients, which is coupled with an H^+ transport in plants. Plants have the ability to change the rhizosphere pH by releasing protons (H^+) or hydroxyl-ions (OH^-) to compensate for an unbalanced cation–anion uptake at the growing medium–root interface (Hinsinger et al. 2003; Riley and Barber 1969). Fluxes of protons (H^+) in the rhizosphere are driven by the fact that the cytosolic pH of 7.3 needs to be maintained (Hinsinger et al. 2003) and for the compensation of negative charges due to an unbalanced uptake of anions versus cations. Nitrogen is an important player, as it is the nutrient taken up in the highest amounts, and it can be taken up as cation, i.e. ammonium ion, or anion, i.e. nitrate. Plants can directly take up amino acids either as a positively charged, neutral or negatively charged (Jones et al. 2005). Plants supplied with nitrate (NO_3^-) will neutralize the negative charges by releasing equivalent amounts of hydroxyl-ion (OH^-) or bicarbonate (HCO_3^-) in the rhizosphere, thereby increasing the pH of the rhizosphere. Plants supplied with ammonium (NH_4^+) will neutralize the excess of positive charges by releasing equivalent amounts of H^+ in the rhizosphere,

thereby decreasing rhizosphere pH (Hinsinger et al. 2003). The pH at the root surface will often differ from the pH in the bulk zone, i.e. a few mm away from the roots, by 1–2 units (Nye 1981) depending on plant and growing medium.

3.3 Microbial nitrogen conversions in soil and soilless culture systems

Growing media, in general, have a low nutrient content, and fertilizers (organic and inorganic) are mainly added to the growing medium to optimize the physico-chemical conditions of the root environment (Sonneveld and Voogt 2009b). The use of organic fertilizers rely on microbial activity and these organic fertilizers have in common that the majority of the nitrogen (N) and, partly, also phosphorus (P) is present in organic form and is released gradually through decomposition by microbial community associated with the growing medium or constituents. We will focus in this thesis on four functional microbial conversions in growing media. The first step is the mineralization of organic derived nitrogen to ammonium. The second step is the oxidation of ammonia (NH_3) to nitrite (NO_2^-), *i.e.* nitritation and subsequently to nitrate (NO_3^-), *i.e.* nitratation. Further reduction of NO_2^-/NO_3^- to N_2 is carried out through denitrification.

3.3.1 Organic nitrogen mineralization

The organic nitrogen is added as an organic fertilizer to the growing medium. The organic nitrogen is made accessible to the plants by the decomposition of it with ammonium as a side product. This pathway, which was described by Jansson (1958), is generally known as the mineralization-immobilization-turnover route. Primarily product of this process is ammonia (NH_3) which depending on the pH of the growing medium directly withdraws an H^+ to form ammonium (NH_4^+). Consequently, an OH^- remains and this results in an increase of the pH of the growing medium.

Microorganisms are also capable of taking up small organic molecules like amino acids (Barak et al. 1990; Geisseler et al. 2010). The fungi (*Alternaria*, *Aspergillus*, *Mucor*, *Penicillium* and *Rhizopus*) and some bacteria are active decomposers of proteins, amino acids and other nitrogen containing compounds (Haynes 2012). Ammonium can also be immobilized by GMC with a cation exchange capacity (CEC), such as clays, which is called the abiotic N immobilization. Plants need oxidized and reduced nitrogen compounds, such as ammonium, nitrate and organic nitrogen compounds. Microorganisms, such as bacteria and fungi, also assimilate these N forms. These microorganisms convert it in organic forms, which are known as the biotic immobilization. The ammonification of amino acids is catalyzed by amino acid dehydrogenases and amino acid oxidases (Eq. 3) (Haynes 2012).

$$\text{Amino-acids (R-NH}_2\text{)} + 1.5\ O_2 \longrightarrow CO_2 + H_2O + NH_3 \qquad \Delta G^{0\prime} = -376\ \text{KJ mol}^{-1} \qquad \text{(Eq. 3)}$$

3.3.2 Nitritation

The second step (Eq. 4-7) is the oxidation of ammonia to nitrite by the ammonia oxidizing bacteria (AOB). The bacteria belonging to the β-and γ-Proteobacteria oxidize ammonia to hydroxylamine (NH_2OH) with the membrane bound enzyme ammonia monooxygenase (AMO). The periplasmatic hydroxylamine oxidoreductase (HAO) then oxidizes hydroxylamine to nitrite. This reaction provides the two reducing equivalents for the first step. The two other produced electrons are used for respiratory purposes, i.e. reducing oxygen (O_2) by a terminal oxidase, thereby generation a proton (H^+) motive force. The isolation of the marine *Nitrosopumulus maritimus* (Könneke et al. 2005) proved that not only AOB, but also aerobic Ammonium Oxidizing Archaea (AOA) are capable of ammonia oxidation.

$$NH_3 + O_2 + 2H^+ + 2e^- \xrightarrow{AMO} NH_2OH + H_2O \quad \text{(Eq. 4)}$$

$$NH_2OH + H_2O \xrightarrow{HAO} NO_2^- + 5H^+ + 4e^- \quad \text{(Eq.5)}$$

$$0.5\ O_2 + 2H^+ + 2e^- \xrightarrow{\text{terminal oxidase}} H_2O \quad \text{(Eq.6)}$$

$$NH_3 + 1.5\ O_2 \longrightarrow NO_2^- + H^+ + H_2O \qquad \Delta G^{0\prime} = -271\ \text{KJ mol}^{-1} \quad \text{(Eq.7)}$$

Archaeal *amo*-like gene sequences were found in oceans, estuaries, soils, and animal gut (Schleper 2010). *Nitrosomonas* and *Nitrobacter* present in peat based materials showed increased activity when the peat was fertilized and limed (Herlihy 1971; 1973). The study of Zhang et al. (2012a) provides strong evidence for autotrophic ammonia oxidation driven by (AOA) rather than AOB in the acidic soils. Peat has a low pH(H_2O). Levičnik-Höfferle et al. (2012) demonstrated that the oxidation of ammonia generated from native soil organic matter or added organic N, but not added inorganic N, was accompanied by increases in abundance of the Thaumarchaeal *amoA* gene, a functional gene for ammonia oxidation. Bacteria are sensitive to increased levels of ammonia (Anthonisen et al. 1976). Different inhibitory ranges for free ammonia (FA) and free unionized nitrous acid (FNA) inhibit nitritation. The concentrations of FA that inhibit *Nitrosomonas* are greater than those that inhibit *Nitrobacter*. The ranges of FA concentrations that show inhibition of the nitrifying organisms is 10 to 150 mg NH_3-N L^{-1} for *Nitrosomonas* and from 0.1 to 1.0 mg NH_3-N L^{-1} for *Nitrobacter*. The inhibition of nitrifying organisms was initiated at concentrations of FNA between 0.22 and 2.8 mg FNA L^{-1} (Anthonisen et al. 1976). The FA and FNA inhibition of nitrification has been shown to occur with municipal, industrial and agricultural wastes and with fertilizers in the soil. All detoxification reactions of ammonium involve the formation of C–N bonds. AOB are not the only bacteria that can oxide ammonia via hydroxylamine to nitrite. A large number of heterotrophic bacteria, among which *Paracoccus pantotrophus* and *Alcaligenes faecalis*, and fungi are capable of this conversion (Wittebolle 2009). It is generally accepted that these organisms do not gain (enough) energy from the conversion of ammonia, as they still need another organic energy source for growth (Blagodatsky et al. 2006). The physiological consequences of heterotrophic nitrification and its importance in various environments remain highly debated (Kowalchuk and Stephen 2001).

3.3.3 Nitratation

The nitrite oxidizing bacteria (NOB) belong either to the Proteobacteria (*Nitrobacter* (α-), *Nitrococcus* (γ-), *Nitrospira* (δ-) and *Nitrotoga* (β-) or to the genus *Nitrospira* of the phylum *Nitrospirae* (Koops and Pommerening-Röser 2001). The third step (Eq. 8-10) is the oxidation of nitrite to nitrate, catalyzed by a nitrite oxidoreductase (NXR) delivering two electrons that are transferred to oxygen with a terminal oxidase (Starkenburg et al. 2011). The oxygen needed for the oxidation of nitrite to nitrate is not delivered by molecular oxygen itself, but originates from water (Aleem et al. 1965; Kumar et al. 1983):

$$NO_2^- + H_2O \xrightarrow{NXR} NO_3^- + 2H^+ + 2e^- \quad \text{(Eq.8)}$$

$$0.5\ O_2 + 2H^+ + 2e^- \xrightarrow{\text{terminal oxidase}} H_2O \quad \text{(Eq.9)}$$

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3 \qquad \Delta G^{0\prime} = -54\ \text{KJ mol}^{-1} \quad \text{(Eq.10)}$$

3.3.4 Denitrification

The denitrification process (Eq.11) is performed by autotrophic or heterotrophic denitrifiers and is a step-wise reduction of nitrate/nitrite to dinitrogen gas (Matějů et al. 1992). Loss of dinitrogen gas in growing media or composting can be a result of denitrification. In organic growing media, heterotrophic denitrifying bacteria are present since adequate amounts of carbon sources are usually present. Heterotrophic denitrifiers reduce nitrate/nitrite according to the following biochemical pathway (Zumft 1997):

$$NO_3^- \xrightarrow{NAR} NO_2^- \xrightarrow{NIR} NO \xrightarrow{NOR} N_2O \xrightarrow{NOS} N_2 \quad \text{(Eq.11)}$$

$\Delta G^{0\prime} = -161$ KJ mol^{-1} $\Delta G^{0\prime} = -76$ KJ mol^{-1} $\Delta G^{0\prime} = -306$ KJ mol^{-} $\Delta G^{0\prime} = -340$ KJ mol^{-}

The enzymes involved in each of these reduction steps are nitrate reductase (NAR), nitrite reductase NIR), nitric oxide reductase (NOR), and nitrous oxide reductase (NOS).

3.4 Nitrogen in relation to the soil and soilless culture systems

Soil and soilless culture systems in combination with growing media are heterogeneous aggregates of different constituents containing solid, liquid and gaseous phases. All of these phases interact with the nutrients and diffusion and mass transfer determine the availability of the nutrients. The inorganic constituents of the solid phase, like clay, provide a reservoir of potassium, calcium, magnesium and iron. The organic compounds of the solid phase contain nitrogen, phosphorus and sulphur, among other elements. The liquid phase is the aqueous solution, which contains dissolved nutrients and serves as the medium for ion movement to the root surface. Gases such as oxygen (O_2), carbon dioxide (CO_2), ammonia (NH_3) and nitrogen gas (N_2) are dissolved in the growing medium solution, but roots exchange gases with the growing medium predominantly through the air gaps between the different constituents. Mineral cations such as ammonium (NH_4^+) and potassium (K^+), adsorb to the negative surface charges of inorganic and organic soil and growing medium particles. This cation adsorption is an important factor in the fertility of the soil. Mineral cations adsorbed on the surface of particles provide a nutrient reserve available to plant roots. The degree to which a growing medium can adsorb and exchange ions is known as its cation exchange capacity (CEC), and is highly dependent on the growing media and the constituents used (Abad et al. 2005; Verhagen 2007). The fixation of ammonium by clay or the organic matter is known as the abiotic nitrogen immobilization.

3.5 N-source preference by plants in relation to the microbial community and soil and soilless culture system

Ammonium and nitrate are the prevalent nitrogen sources for growth and development of higher plants. From an energetic point of view, ammonium uptake and assimilation are less costly than nitrate uptake and assimilation, indicating a competitive advantage for plants with a high ammonium absorption capacity. However, ammonium can cause severe toxicity symptoms (Britto and Kronzucker 2002) and *Solanaceae* are well known for its high ammonium sensitivity. By contrast, nitrate toxicity is uncommon. This ammonium toxicity may jeopardize the energetic advantage in taking up ammonium rather than nitrate. Moreover, next to nitrogen, plants need large amounts of potassium, calcium, magnesium, and other cations. Consequently, the absorption of nitrate may lead to a more even charge balance for plants than the absorption of ammonium. Furthermore, ammonium is known for its abiotic immobilization by soil and organic growing media, while nitrate is highly mobile it can lead

to leaching losses. The high mobility of nitrate, leads also to a rapid diffusion of nitrate to the roots and consequently it is more accessible for plants (Boudsocq et al. 2012).

One of the most important factor affecting ammonium or nitrate uptake is soil temperature (Britto and Kronzucker 2013). Nitrate uptake appears to be more limited by low temperature than does ammonium uptake, which can be explained by its higher energy requirement for acquisition and reduction (Frota and Tucker 1972). In general, plants adapted to growing media or soils, which have a low pH or have a low redox potential, such as wetlands have a preference for ammonium (Lee 1998). In contrast, plants adapted to growing media with a high pH utilize nitrate preferentially. ^{15}N-uptake studies demonstrated that ammonium is preferred up to 20-fold over nitrate by Arabidopsis plants, when ammonium and nitrate are supplied in equimolar ratio's, particularly when the N supply is low (Gazzarrini et al. 1999). The acidity of the soil or growing medium affects nitrate and ammonium transport differentially (Haynes and Goh 1978). The uptake of NO_3^- requires a higher amount of protons than for NH_4^+, since it is driven by a proton-symport mechanism, while NH_4^+ uptake probably occurs in a uniport manner, in exchange for protons under most conditions (Britto and Kronzucker 2013). At very low concentrations, NH_4^+ uptake involves a symport mechanism with protons (Ortiz-Ramirez et al. 2011), while at high concentrations NH_3 permeation may be possible through aquaporins (Jahn et al. 2004). The mechanisms of transport for the two ions themselves have direct impact for soil or growing medium acidity as described before. These processes can result in unpredictable feedback cycles that can be intensified by other factors. For example, inhibition of nitrification rates by the microbial community associated with the growing medium as the rhizosphere acidifies (Falkengren-Grerup 1995; Haynes and Goh 1978) and changes in the availability of nutrients, such as phosphorus, or toxicants, as soil pH changes differentially depending on the N source dominates in plant acquisition patterns (Britto and Kronzucker 2013).

Plants are able to assimilate different kind of nitrogen forms, such as organic nitrogen, ammonium and nitrate. In addition, mineralization of the organic nitrogen results in a release of ammonium and depends on the mineralization rate of the organic nitrogen. Ammonium is then converted in nitrate during nitrification, so the abundance of nitrate depends on both the abundance of ammonium and the ammonia and nitrite oxidation rate (Boudsocq et al. 2012). Moreover, the competition between plants and microorganisms can also be stronger on ammonium than nitrate (Hodge et al. 2000).

Some plants are able to inhibit (Lata et al. 1999; Lata et al. 2000; Lata et al. 2004; Subbarao et al. 2007a) or stimulate (Hawkes et al. 2005; Lata et al. 2000) nitrification, thereby shifting the relative amount of ammonium and nitrate available in the soil or growing medium for their own mineral N nutrition, as well as for the mineral N nutrition of their competitors. The inhibition of nitrification may increase primary productivity and soil or growing medium fertility. Indeed, inhibiting nitrification enhances the conservation of ammonium in the soil or growing medium, as nitrate is more prone than ammonium to being lost from the ecosystem. This could be of particular importance in poor or well-draining soils or growing media. It has also been hypothesized that nitrification-inhibiting plants may also have developed a greater capacity for the absorption of ammonium than for nitrate (Boudsocq et al. 2009). Immobilization of nitrogen by microorganisms does not affect the availability of ammonium and nitrate for plants at equilibrium stage (Boudsocq et al. 2012).

The reasons for the preference for ammonium or nitrate or even organic nitrogen are poorly understood (Boudsocq et al. 2012), moreover, nitrogen conversion in the soil and soilless culture

systems are highly dynamic making it even more complex and unpredictable. A large knowledge gap exists concerning the nitrogen uptake preferences as a function of the cultivating system, i.e. soil or soilless culture systems, the use of organic or inorganic fertilizers, the plant age and the microbial community composition.

4. Soil and soilless culture systems in relation to the microbial community ecology

The microbiota of peat, which is the most important growing medium constituent (Schmilewski 2007), consists of a small number of genera and species (Dickinson and Dooley 1967; Küster and Locci 1963; Walsh and Barry 1957). Most of the organisms present in peat originating from peat bogs are in a dormant or resting state, which is translated into a low microbial activity (Carlile and Wilson 1990). Processing of the peat improves the aeration through sieving and other physical factors (Dickinson and Dooley 1967), resulting in increased microbial concentrations and activity. The quantitative and qualitative composition of the microbiota of milled peat depends largely on the content of available nutrients and energy sources. The more decomposed, humified and carbonized the carbon material in peat, the smaller the content of utilizable compounds and the less available these become for the microorganisms present (Küster 1971). Peat-based materials with a low degree of decomposition still contain organic matter, which can be used by microorganisms. The less-decomposed layers contain more hemicelluloses and celluloses than the well-decomposed ones (Lindberg and Theander 1952; Theander 1954; Waksman and Stevens 1928). Yeasts and Actinobacteria and Bacteroidetes seem to play an important role during the decomposition of organic material (Boehm et al. 1993; Thormann et al. 2007). Other organic growing media constituents are colonized by a wide variety of microorganisms. Composted bark another important growing media constituent seems to be primarily colonized by fungi (Carlile and Wilson 1990). During the composting of bark potentially toxic organic substances could be destroyed or, in contrast, even accumulated, such as phenolic compounds (Erhart et al. 1999). The composting process may also eliminate other beneficial bacteria (Carlile and Wilson 1990).

Another important growing media constituent is green waste compost. During the various composting stages, compost is colonized by different microbial communities, each of which being adapted to a particular environment (Ryckeboer et al. 2003b). Primary decomposers build a physico-chemical environment appropriate for secondary organisms, which cannot attack the initial nutrients, while metabolites produced by the one group can be utilized by the other (Davis et al. 1992). The initial rapid increase of temperature involves a rapid transition from mesophilic to thermophilic microbial community (Corominas et al. 1987; Niese 1959; Ryckeboer et al. 2003a).

Inorganic growing media and constituents, such as perlite, vermiculite and mineral growing media are on the contrary sterile prior to their use. The primary source of micro-organisms is coming from transplants, handling processes, fertigation and aerial transmission (Carlile and Wilson 1990). The colonization of the mineral growing media constituents occurs in the presence of the plant when the roots are growing into the growing media. The diverse and competitive saprophytic microbial community associated with the organic growing media and their constituents (Donnan 1998; Olle et al. 2012) can influence the nutrient status of the root environment (Carlile and Wilson 1990). The numbers of microorganisms in soilless culture systems are typically low at the start of a crop, and so is the diversity (Postma et al. 2000).

With the development of modern and molecular techniques a number of studies monitored the community dynamics in soil and explored interactions of microbial species with abiotic factors (Dumbrell et al. 2010; Frostegård et al. 1993). While progress is made understanding the microbial ecology in soil, a large knowledge gap exists concerning the soilless microbial ecology, their structure, dynamics and sensitivity towards the use of mineral and organic fertilizers.

4.1 Microbial community composition in relation to biodiversity

The term 'biodiversity' is a contraction of 'biological diversity' and is defined as the sum of all biotic variation from the level of genes to ecosystems (Purvis and Hector 2000). Species richness and species evenness are used to describe biodiversity. The most frequently studied feature of biodiversity is species richness or the number of different species in a habitat (Balvanera et al. 2006; Purvis and Hector 2000). Darwin and Wallace (1858) stated that a diverse mixture of plants should be more productive than a monoculture (Darwin & Wallace, 1858) and this is still valid until today. The reason for this increased productivity is explained by the large variety of positive and complementary interactions between the different species (Tilman 1999). Species evenness is a parameter that describes how well distributed the abundance of species is within a community by counting the number of individuals per species. Species richness can also impact ecological processes such as the resistance of communities to invasion like pathogen. Research that investigates microbial community ecology (species evenness) in soil and especially in soilless culture systems in relation to productivity is scarce.

The activity and diversity of soil bacterial communities are directly influenced by the physico-chemical properties (Wu et al. 2011) and most likely this is also the case for soilless culture systems. A significant relationship between soil bacterial communities and soil water content was observed in a replicated field trial with winter flooding effects (Bossio and Scow 1998). Bååth (1996) differentiated soil bacterial communities of 16 different soils with pH(H_2O) ranging between 4 and 8. Schutter et al. (2001) indicated that although seasonal and field-dependent factors are major determinants of microbial community structure, shifts occurred as soil physical and chemical properties change in response to farm management practices. Anthropogenic activities including agricultural land management practices directly and indirectly affect soil physical and chemical properties and thus may also alter the activity and diversity of soil bacterial communities (Wu et al. 2011). The effects of agricultural land management practices on soil microbial communities have been widely studied using culture-based methods that include dilution plating and sole carbon source utilization and biochemical biomarkers. Organic amendments were shown to significantly enhance populations of soil bacteria antagonistic to plant pathogens in the rhizosphere of tomato (De Brito et al. 1995). The source of fertilizer used (inorganic, organic or decomposed plant material) had a larger effect on microbial communities, where PLFA's were used as an index of diversity, than land management systems (organic, low-input, or conventional) (Bossio and Scow 1998). In a study on soil bacteria from an agricultural tomato field, divided according to five different land management programs, it was detected that differences in bacterial diversity were attributed to differences in evenness and not in richness (Wu et al. 2008).

4.2 Microbial community analysis

Many microbial communities are complex and consist of very different microorganisms. There are several techniques (Figure 1.7) available for investigation the microbial community and they are divided in two general types of techniques: culture dependent and culture independent (Rosberg

2014). Plate counts are an example of culture dependent techniques and are still the most used technique. Samples from different environments can be grown on solid and in liquid nutrient media. However, fast and slow growing microorganisms appear both together in nature. When the fast growing microorganisms are brought onto a nutrient medium the fast growing microorganisms, will outgrow the low growing microorganisms and therefor will give a wrong impression of the microbial community composition in an environment. Moreover, different microorganisms require different nutrient and growth conditions. The main problem associated with cultivation methods is that the plate counts obtained by cultivation (colony forming units, CFU) are usually much lower than those from direct cell counts under a microscope, i.e. only 0.1-1% of all the microorganisms will be revealed by the plate count method (Madigan et al. 2008; Torsvik et al. 1990). In addition, the activity of the microbial community can be determined by measuring *in situ* and *ex situ*, such as the respiration, ammonia oxidation and nitrite oxidation rate. However, based on these methods nothing can be concluded on the microbial community composition.

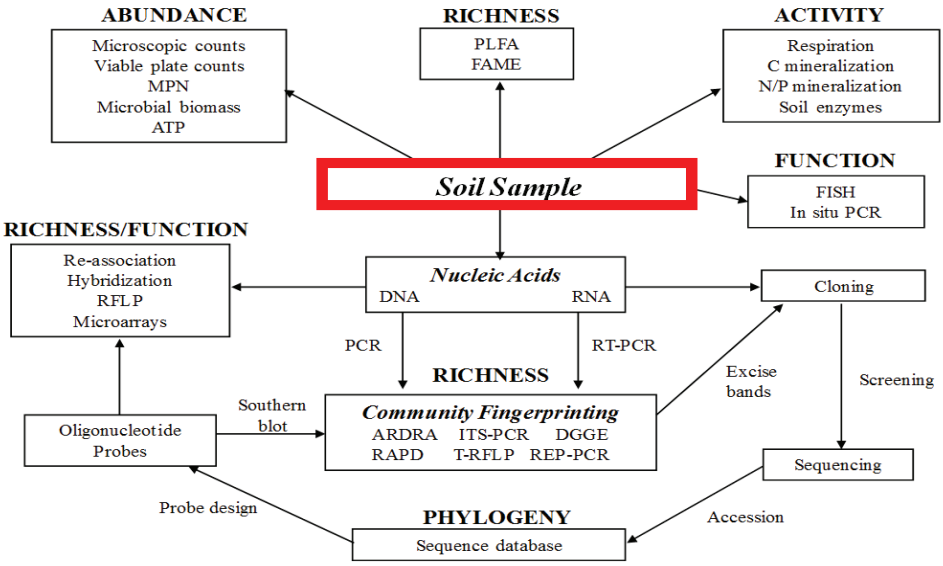


Figure 1.7: Overview of methods to study the microbial community in growing media and the soil according to Paul (2006)

Despite the recent progress in cultivating microorganisms, these methods are considered unreliable and inadequate to describe the entire microbial community. This problem is circumvented by combining culture dependent and independent techniques (Figure 1.8) based on the DNA/RNA structure, thus without the unreliable culturing steps and the non-DNA structure (Edenborn and Sexstone 2007; Shade et al. 2012). Non-DNA based techniques are for instance phospholipid-derived fatty acids (PLFA) analysis, that are widely used in microbial ecology as markers of bacteria and other organisms (Buchan et al. 2012; Khalil and Alsanius 2001). The presence of specific fatty acids admits the abundance of specific groups of microorganisms. The quantification of microorganisms in an environment can be done by culture dependent techniques, however it can also be done by the so

called “quantitative PCR (qPCR)” (Wittebolle 2009). By using a Sybr Green DNA dye or a Taqman probe, the amplification can be tracked in real time by measuring the amplicon concentration. Real time PCR analyzes the relative abundance of PCR products during the exponential phase, in which reagents are not limited. Real time PCR allows to compare the transcript abundance between two different samples, since the PCR product quantity in the exponential phase corresponds with the initial template abundance. The relative quantification can be achieved by analyzing the so-called Ct value.

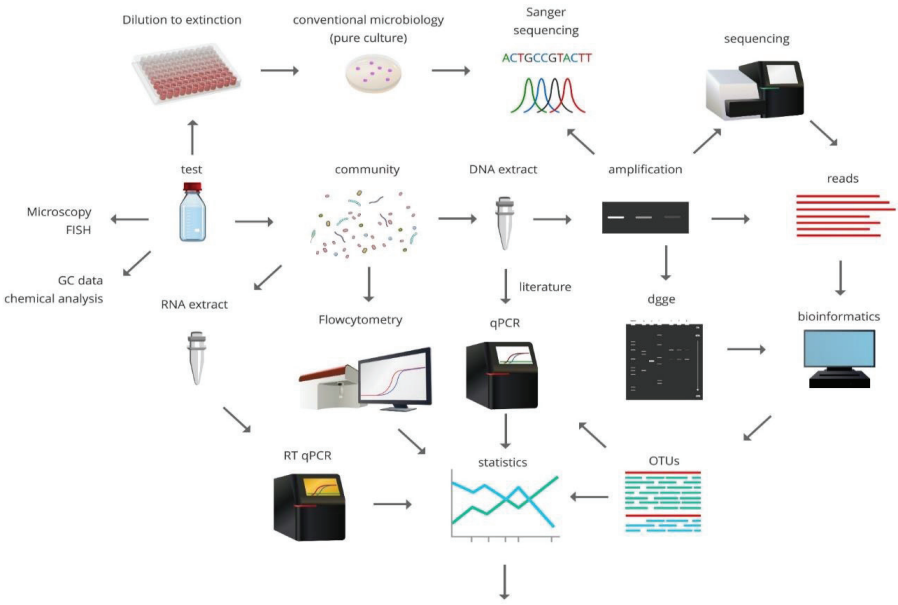


Figure 1.8: Overview of molecular techniques to study the microbial community in a sample – starting from a sample in a Schott bottle. Drafted by Tim Lacoere (2016)

Another DNA based method is DGGE (Denaturing gradient gel electrophoresis), which gives a specific pattern of the microbial community (Boon et al. 2002; Calvo-Bado et al. 2006). This method was developed for the detection of specific DNA mutations, i.e. DNA fragments of the same length but with a different base pare sequence, which are separated on a denaturing gel. This technique allowed to characterize the structure of a microbial community by studying the profile of the amplified 16S rRNA from the DNA of the microbial community. From the DNA of the microbial community, a well specified region of the 16S rRNA will be amplified with PCR by using specific primers. Due to these denaturants the double DNA strands of the same length can be separated based on their basepare sequence or more specifically depending on the GC content of the DNA. The community profiles from different samples can be compared and the level of similarity can be calculated.

Illumina and 454 pyro sequencing technologies have recently been recognized for their applicability in profiling complex microbial systems and the drop in price. The Illumina system is a sequencing by-synthesis method depending on detection of fluorescent, reversible terminator nucleotides, and 454 pyrosequencing, is a nucleotide based incorporation detection method via pyrophosphate release and luciferase activity, that facilitate simultaneous sequencing and enumeration of every amplified transcript in a mixed sample (Bokulich et al. 2012). The generation of several million sequence libraries from variable-region amplicons (usually a hypervariable domain of prokaryotic 16S rRNA genes) enables the characterization of the complete microbial community in a given sample. This method, as with all PCR-based methods, is limited by a number of issues: PCR primer amplification bias (Polz and Cavanaugh 1998) and DNA extraction bias (Cocolin et al. 2001) may influence the apparent community composition. Compared with DGGE, pyrosequencing and Illumina provide much more sensitive, relative quantification, with streamlined input to statistical comparisons among samples. Illumina sequencing platforms are particularly suited to studying complex microbial systems, such as growing media (Grunert et al. 2016a) and soil.

4.3 Ecological measurements of diversity

To evaluate data from next generation sequencing, such as Illumina, ecological measurements of diversity can help in describing the community of interest, as well as community composition both in quantitative and qualitative way. Diversity is defined as a measure of how much variety is present in a community, irrespective of the identities of the organisms. Measuring diversity is important for understanding community structure and dynamics (Lozupone and Knight 2008) and there are many diversity indices (Table 1.2) trying to refine that information. It has been agreed that species evenness and richness are two aspects contributing to the intuitive concept of diversity. Species richness simply measures total number of species in the community, while evenness describes how evenly the individuals are distributed over the different species in the community (Heip and Engels 1974).

Many indices that have been created to measure diversity vary in the particular aspect of diversity that they measure, their sensitivity to different abundance classes and their failings (Table 1.2). In this thesis, richness, Pielou's index for species evenness, and four indices of diversity (Shannon, Simpson, Inverse Simpson, and Fisher's) were calculated. The Shannon index, which is the negative sum of each species proportional abundance multiplied by the log of its proportional abundance, is a measure of the amount of information (entropy) in the system and hence is a measure of the difficulty in predicting the identity of the next individual sampled. Simpson's index (D) gives a strong weighting to the dominants. It is also easily understood and it gives the probability that two observations chosen at random will be from the same species.

The diversity measures and abundance models vary in their usefulness with such diverse communities. Some are more suited for use with limited-coverage datasets while others are for use with larger samples that provide at least 50% coverage of species. As a result, we calculated all the diversity indices, to account for sensitivity differences among indices. Besides looking at diversity indices, relative abundances of each species is valuable since they describe the proportions in which each species are present in the community. Species abundance models are useful because they address the overall distribution of a sample, assisting comparison by revealing whole trends and specific changes in particular abundance classes. They are also more sophisticated to investigate diversity because they

examine the distribution of abundances in a population rather than distilling all this information down into a single number (Hill et al. 2003).

Table 1.2: Difference between measured diversity indices

Metric	Formula	Aim
Richness (S)	Number of species	Simple measure of how many different species the dataset of interest contains
Shannon's diversity (H')	$\sum P_i \ln(P_i)$	H' is equally sensitive to rare and abundant species; sensitivity to rare species increases as a decreases from 1, and sensitivity to abundant species increases as a increases from 1
Simpson's diversity (D_1)	$1- \sum P_i^2$	Measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species). Sensitive to abundant species
Simpson's dominance (D2) or Inverse Simpson diversity	$1/\sum P_i^2$ where p_i is the proportion of all individuals belonging to the i_{th} species	Used to quantify average proportional abundance of species in the dataset of interest
Fisher's Alpha (α) diversity	$S=a*ln(1+n/a)$ where S is number of taxa, n is number of individuals and a is the Fisher's alpha	Widely used as a diversity index to compare among communities varying in number of individuals (N), because theoretically independent of sample size
Pielou's evenness	$J = H'/\ln(S)$ where H' is Shannon Weiner diversity and S is the total number of species in a sample, across all samples in dataset	Measure of the relative abundance of the different species making up the richness of an area

5. Thesis outline and the research questions

This thesis deals with the increased demand for novel soilless culture systems in combination with organic growing media. These organic growing media are used in the highly competitive fresh vegetable market as a result of the increasing claim of retailers and society to find solutions for the vast volumes of waste that needs to be recycled every year and the use of inorganic fertilizers and biocides that are needed to guarantee final yield and quality. The main objective of this study was to shed a light on the microbial community ecology in soilless culture systems, to quantify key functionalities (respiration, ammonia and nitrite oxidation rate) of individual growing medium constituents and to figure out the N dynamics in relation to the microbial community associated with the growing medium and the plant. Growing media are major constituents of soilless culture systems and in this thesis, two different growing media were used throughout this thesis: a mineral (RW) and an organic growing medium (GB). An overview of the different research chapters is given in Figure 1.9.

The main hypotheses (H) of this study were:
H1: The organic growing medium (GB) has a higher species diversity compared to a mineral growing medium (RW).

H2: In contrast to the individual medium constituents, a blend creates a more optimal physico-chemical microbial environment, resulting in higher ammonia and nitrite oxidation rates.
H3: In tomato soilless cultivation systems, a gradual increase of the organic nitrogen supply rate results in comparable yields compared to inorganic fertilizers.
H4: Novel recovered fertilizers can replace conventional fertilizers resulting in a comparable plant performance (yield and quality).

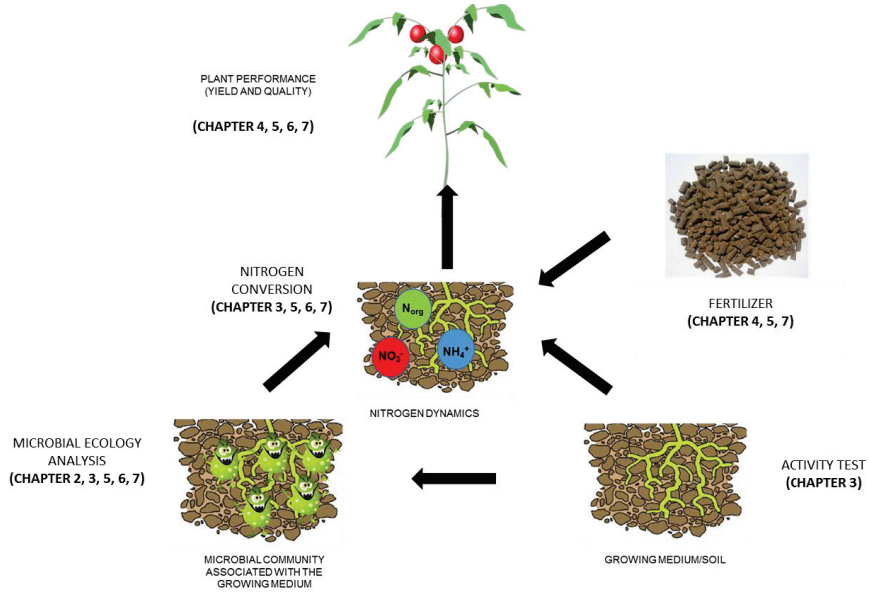


Figure 1.9: Overview of the different research chapters

As this thesis is done in close cooperation with the growing media industry, it was also expected to:

- identify critical parameters with respect to internal quality control of these growing media
- find solutions for the high ammonium/ammonia concentrations in growing media blended with organic fertilizer resulting in too high pH values
- develop novel soilless culture systems for the production of vegetables in combination with the predefined organic growing medium (GB), which was used throughout all the tests.

Hypothesis H1 is addressed in **chapter 2**, where the microbial community composition was examined in the two most important growing media (organic and mineral) in a closed conventional soilless greenhouse culturing system. In **chapter 6** we examined the microbial community of four different open tomato cultivating systems and in **chapter 7** the rhizospheric microbial community composition and the microbial community composition in the bulk zone of a tomato plant and lupine. By comparing the microbial community composition in the different experimental set-ups and the determination of the physico-chemical characteristics, the following research questions were answered.

RQ1: 'Do organic growing media have a higher species richness, diversity, and evenness compared to mineral growing media in closed soilless culture systems?

RQ2: What are the differences in microbial community composition (species richness, diversity and evenness) in a mineral growing medium with plants showing the hairy roots syndrome and plants not showing the hairy roots syndrome?

RQ3: What are the differences in microbial community composition between the rhizosphere and the bulk zone?

RQ4: Are plants rather than fertilizers drivers of the microbial community composition in organic growing media blended with recovered nutrients?

RQ5: Do soilless culture systems with organic fertilizer and organic growing media have a higher species richness, diversity, and evenness compared to other contrasting tomato cultivation systems (GBFISH, SOILANIMAL and SOILPLANT)?

RQ6: What are the most significant physico-chemical characteristics of the soil and soilless culture systems, i.e. mineral and organic growing medium correlated with the microbial community.

Chapter 3 investigates the activity, i.e. the respiration, ureolytic, ammonia and nitrite oxidation rate of a predefined organic growing medium (GB) and its growing medium constituents and thus addressed hypothesis H2. Besides the determination of the respiration, ureolytic, ammonia and nitrite oxidation rates of the predefined blend (GB) and its individual GMC, **chapter 3** deals with the outcome of an in practice simulated lab test related to nitrogen dynamics and changes in the relative abundance of bacterial *amoA* genes and the total bacteria. Therefore, the following research questions were formulated:

RQ7: Do we see an inhibition, a status quo or a stimulation of the respiration, ureolytic, ammonia and nitrite oxidation rate when individual GMC are blended with each other?

RQ8: What are the critical quality control factors with respect to the use of organic fertilizers blended into a growing medium?

RQ9: Can we use commercial available liquid organic fertilizers and to what extent do we need to adapt the N fertilization strategy and can we estimate the risk of ammonium toxicity.

Chapter 5 deals with the development of a fertigation strategy in combination with organic fertilizers and organic growing media. This chapter addresses H3. One research question was derived from H3:

RQ10: What is the effect of a gradual increase of the organic nitrogen supply rate on the yield and the quality of the tomatoes compared to a constant inorganic nitrogen supply rate?

Chapter 4, **chapter 5**, **chapter 6** and **chapter 7** deal with several commercial available organic and novel fertilizers, such as ammonium struvite and microalgae and organics, such as blood meal and malt sprouts and their effect on N conversion dynamics. These chapters collectively address hypothesis (H4). Four research questions were derived from H4:

RQ11: What is the effect of organic fertilizers, fertilizers based on microalgae (MaB flocs and Nannochloropsis), fertilizers based on plant and animal products and ammonium struvite on the nitrogen conversion dynamics in the soil and soilless culture system?

RQ12: What is the effect of these recovered nutrients on plant performance (yield and quality)?

RQ13: Is it economically feasible to use microalgae in soilless culture systems as a substitute for inorganic fertilizers?

RQ14: How are (organic) soil and soilless culture systems regulated at national and European level? How is the use of recovered nutrients, such as ammonium struvite, regulated at national and European level?

To answer these questions, several preliminary and final plant test were conducted in 2013, 2014 and 2015. Moreover, rhizotron experiments were performed with young tomato and lupine plants to assess the below and aboveground plant performance. Planar optodes helped to quantify the pH dynamics in the rhizosphere and bulk zone in a non-invasive way over time. Moreover, an economic assessment concerning the use of microalgae, and recovered nutrients such as organic fertilizer and struvite and a legal assessment (organic) soil and soilless culture system has been prepared. **Chapter 8** presents a general discussion of the results obtained during this PhD, as well as perspectives for future research.

CHAPTER 2: MINERAL AND ORGANIC GROWING MEDIA HAVE DISTINCT COMMUNITY STRUCTURE, STABILITY AND FUNCTIONALITY IN SOILLESS CULTURE SYSTEMS

This chapter has been redrafted after:

Oliver Grunert, Emma Hernandez-Sanabria, Ramiro Vilchez-Vargas, Ruy Jauregui, Dietmar H. Pieper, Maaike Perneel, Marie-Christine Van Labeke, Dirk Reheul, and Nico Boon. "Mineral and organic growing media have distinct community structure, stability and functionality in soilless culture systems." Scientific reports 6 (2016).

ABSTRACT

In horticultural systems, the choice of soilless growing medium for plant nutrition, growth and support is crucial for improving the eco-sustainability of the production. As our current understanding of the functional microbial communities inhabiting this ecosystem is still limited, we examined the microbial community development of the two most important growing media (organic and mineral) used in closed soilless horticultural systems. We aimed to identify factors that influence community composition over time, and to compare the distribution of individual taxa across growing media, and their potential functionality, i.e. stability. High throughput sequencing analysis revealed a distinctive and stable microbial community in the organic growing medium. Water content, pH(H_2O), nitrate-N, ammonium-N and conductivity were uncovered as the main factors associated with the resident bacterial communities. Ammonium-N was correlated with *Rhizobiaceae* abundance, while potential competitive interactions between both *Methylophilaceae* and *Actinobacteridae* with *Rhizobiaceae* were suggested. Our results revealed that soilless growing media are unique niches for diverse bacterial communities with temporal functional stability, which may possibly affect the resistance to external forces. These differences in communities can be used to develop strategies to move towards a sustainable horticulture with increased productivity and quality.

1. Introduction

In the U.S., Canada and Europe, 95% of glasshouse vegetables, particularly tomatoes, are produced in soilless glasshouse plant cultivation systems using horticultural growing media (Peet and Welles 2005). Open soilless horticultural systems have advantages over traditional systems in that the nutrients, oxygen and water required for a healthy plant growth are controlled (Raviv et al. 2008) and that soil-borne pathogens can be circumvented (Postma 2009; Runia 1993). In Western Europe, nearly all glasshouse-grown tomatoes are produced on mineral growing medium comprised of inorganic synthetic fibers (Islam 2008). Rockwool, the most often used mineral growing medium, is produced from diabase, limestone and cokes, which are melted together at 1500°C and spun into fibers (Pluimers 2001). In contrast, peat and coconut are the most utilized organic-derived constituents of growing media produced in the EU (Altmann 2008). While the mineral growing medium has a neutral pH(H_2O), high air content and low dry bulk density, the organic growing medium is characterized by its high organic matter content and capacity for cation exchange with the water solution irrigating the growing medium. In spite of these differences, the yield and number of tomato fruits (*Solanum lycopersicum*) was comparable among plants grown on either mineral or organic growing media over several consecutive years (Grunert et al. 2008).

Soilless culture systems rely heavily on increased yields and on the general efficacy of the growing process (Postma 2009). Disinfection measures are taken at the glasshouse to guarantee final yield and quality (Postma 2009). However, this results in the elimination of not only deleterious microorganisms but also of potentially beneficial microbial taxa for the plant. This may ultimately prevent the community from reaching equilibrium and stability, making these soilless cultivation systems at risk of successful pathogen invasion (Postma 2009). Differences in terms of the microbiota inhabiting organic and mineral growing media were identified by (Khalil and Alsanius 2001; Koohakan et al. (2004)). They found that bacteria mainly colonized mineral growing media, while the organic growing medium had a larger fungal population. If an organic growing medium is used this could also act as a supplier of carbon. However, little is known about the microbial community composition and structure. Biodiversity protects ecosystems against declines in their functionality, as a consequence of the functional redundancy through the co-existence of multiple species (Balvanera et al. 2006; Yachi and Loreau 1999). This can also lead to increased productivity (McCann 2000), due to positive impact on bacterial respiration, microbial biomass production, and plant nutrient storage. In addition, increased temporal functional stability and resistance to external forces, such as nutrient perturbations and invasive species, have been reported (Balvanera et al. 2006; Wittebolle 2009).

The complex plant-associated microbial community, also referred to as the “second genome of the plant”, is crucial for plant health, growth and development (Raaijmakers 2015). As shown by Rosberg (2014), root-associated and nutrient solution-associated microbial community structures are affected by plant age, pathogen attack, the availability of organic and inorganic nutrients, and the use of plant protection products. Plant age in particular seemed to have the greatest impact on community structure. Previous work investigating microbial communities associated with growing media has mainly focused on the absence of pathogenic bacteria and fungi (Mendes et al. 2011). There is limited understanding of the factors that influence community composition over time, the distribution of individual taxa across growing media, and their potential functionality. The lack of effective control strategies aiming at enhancing productivity (Weller et al. 2002) increases our need to closely monitor the rhizosphere, the growing medium and its microbial populations.

In this study, we examined the microbial community ecology of the two most important growing media used in open soilless culture systems: organic (GB) and mineral (RW). We hypothesized that mineral (RW) and organic (GB) growing media in a closed soilless culture system develop a distinctly different community structure with respect to species richness, diversity, evenness and resistance against *Agrobacterium rhizogenes*. Knowledge regarding these differences can be used to develop strategies towards sustainable horticulture, with enhanced productivity and quality, and potentially increased resistance to external forces (Wittebolle 2009). The hairy roots syndrome caused by *Agrobacterium rhizogenes* infection is a major issue in glasshouse horticulture, because total yield may decrease up to 10% in tomato plants (Van Calenberge 2013). In our study, naturally occurring *A. rhizogenes* infection was detected in some plants growing in the RW medium (RWS). The interactions occurring between the roots, rhizosphere, the growing medium and the potential resistance to external forces, represented in our study by *A. rhizogenes*, were also assessed.

2. Materials and methods

2.1 Experimental setting and growing media

The microbial community associated with the different growing media was monitored in a commercial 8.5 ha glasshouse in The Netherlands (51°59' Latitude and 4°10'Longitude), cultivating the eggplant *Solanum melongena* cultivar Jaylo (Rijk Zwaan, The Netherlands), grafted on root stock of a tomato plant *Solanum lycopersicum* L. x *Solanum habrochaites* Beaufort (De Ruiter, The Netherlands). The glasshouse was a closed system, the drainage is collected, mixed with fresh water, readjusted to the accurate nutrient composition and redistributed to the plants. The two different horticultural growing media were installed at the same time in the glasshouse and the 48-day-old eggplants were planted on top of the two different growing media on the same day. The organic growing medium (GB, Grow Bag, Peltracom, Belgium) was a mixture of Sod peat (H2-H4 on the von Post scale (Von Post 1926) [40% v/v], Irish peat [40% v/v] and coconut fiber [20% v/v]). Slabs of GB and mineral medium (RW, Rock wool, Grotop expert, Grodan, The Netherlands) had the following dimensions: 1.0 m × 0.2 m × 0.085 m and 1.0 m × 0.2 m × 0.075 m, respectively. Both growing media were subjected to identical water and fertilizer treatments during the cultivation period according to standard methods, with standard fertigation solution (Sonneveld and Voogt 2009b). Two eggplants per slab were planted. Each plant was trained to three stems, aiming at a plant density of 1.7 plants m^{-2} resulting in 5.1 stems m^{-2}.

2.2 Sample collection

The glasshouse was divided into several blocks each consisting of 6 rows in a randomized block design (Figure 2.1). Two contiguous blocks were randomly selected and each block contained either RW or GB medium. The two outer rows of each block were not selected, because of possible interactions with the adjacent rows. The eggplants were growing in slabs placed consecutively with an interspacing of 44 cm. One slab was considered an experimental unit. Five slabs from each block were randomly selected from the four inner rows and from the two different growing media (GB and RW). Samples of the different experimental units were collected at three time points during the growing season (June, July and August) and at the start of the experiment. Ten samples from each experimental unit were collected, pooled, homogenized and treated as a single sample (Figure 2.1).

At each time point, samples were taken from 5 fixed experimental units of each RW and GB, including root material. Each sample of 200 g was divided into 4 homogenous samples of 50 g for further analysis. Out of this homogenous sample, two samples (sample 1 and 2) were used for chemical analyzes. One sample (sample 3) was stored at 4°C and used for isolation and identification of Agrobacterium sp. and total CFU, as well as water content determination. Sample 4 was immediately stored on dry ice, preserved at -80°C and used for molecular microbial community analysis.

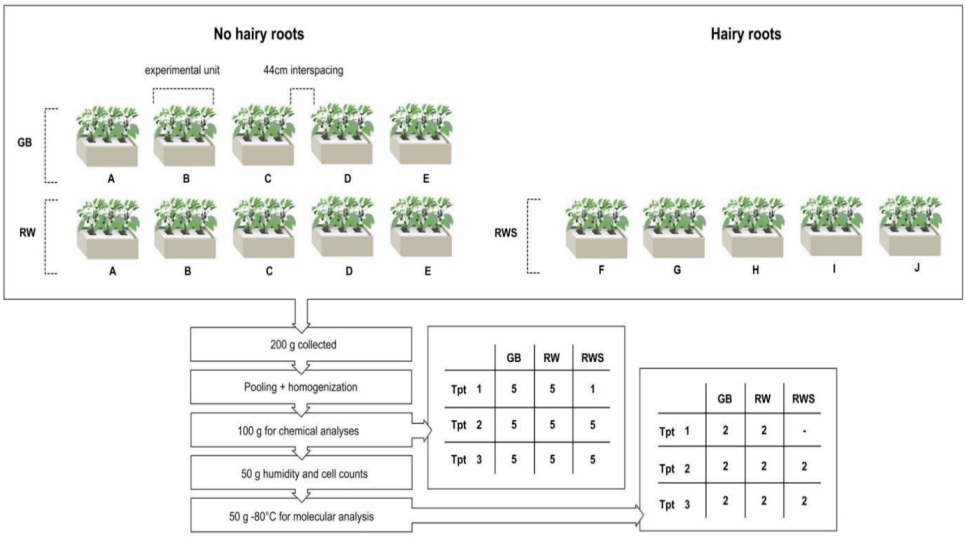


Figure 2.1: Sample collection and analysis procedure. Two different horticultural growing media were selected for investigating their differences in bacterial communities. One type was constituted by organic material (GB) while other was made of mineral fibers (RW). One slab was considered an experimental unit. Incidentally, plants on RW got naturally infected with the hairy roots (caused by Agrobacterium sp.) and those slabs were also sampled (RWS). Ten samples taken at different locations were collected from each slab, pooled, homogenized and treated as a single sample. Samples were taken from 5 fixed slabs of each RW and GB (A, B, C, D and E). Each sample was divided into four individual samples: two were used for chemical analyzes, one for isolation and identification of Agrobacterium sp. and total CFU, as well as water content determination, and sample 4 was used for microbial community analysis. Therefore, five samples of GB were stored at times 1, 2 and 3 (n = 15). None was from sick plants. Five samples of RW without hairy roots at times 1, 2 and 3 were collected (n = 15). Plants in RW started to show hairy roots at the time point 2. Therefore, five samples of RWS (F, G, H, I and J) were obtained at time points 2 and 3 (n= 10). Hence, forty samples were used for determination of physical and chemical characteristics. Illumina sequencing was performed in randomly selected, representative samples as follows: from GB and RW, two samples from each time point (GB sequenced = 6, RW sequenced = 6) and two from plants with hairy roots (RWS) at the second and third time points (RWS sequenced = 4).

The grower reported previous presence of the hairy roots syndrome, which is caused by the pathogen *Agrobacterium rhizogenes*. Hence, disease incidence of the hairy roots syndrome was followed up by a monthly visual inspection of the glasshouse. The hairy roots syndrome was detected in one RW slab

at the first time point in June. Further visual inspection during July and August revealed increased incidence of the hairy roots syndrome in RW medium. Additional samples of RW from 5 additional slabs showing visual symptoms of the hairy roots syndrome were taken (named RWS). However, no hairy roots were visually identified in the GB throughout the entire experimental period (December 2012 and November 2013).

2.3 The physico-chemical analysis of the growing medium

The physicochemical characteristics of the different growing media were determined at the start (December 2012) and during the growing season (June, July and August 2013). The chemical analysis was performed as described by Gabriels et al. (1998b). Potassium, phosphorus, calcium, magnesium, iron and manganese were extracted (1:5 vol/vol) in ammonium acetate and measured with ICP. The electrical conductivity (EC), pH(H2O), ammonium (NH_4^+), nitrate (NO_3^-), sulphate (SO_4^{2-}) and sodium (Na) were measured in a 1:5 v/v water extract according to EN 13038, EN 13037 and EN 13652, respectively. Nitrate was measured with an IC ion chromatograph. Ammonium was measured by steam distillation. The water content of the growing media was determined according to Verdonck and Gabriels (1992). The determination of dry matter content was done according to EN 13040, the determination of organic matter content according EN 13039.

2.4 Determination of the water retention curve of the growing medium

The soil water retention curve of the RW and the GB media was established using the sand box apparatus (DIN 2012) for pressure potentials between -1kPa and -10 kPa. For this experiment, 10 replicates of the slab samples were used.

2.5 Isolation, identification and determination of the Agrobacterium sp. and total cell count

Growing medium was analyzed within 48 hours of sample collection. Five grams of the fresh growing medium were mixed with 45 ml of 0.85% NaCl (Opelt and Berg 2004) and homogenized for 2 minutes, using a Stomacher80 blender (Stomacher, Seward, Worthing, UK). This suspension was used for the determination of the total cell and *Agrobacterium* sp. count on each medium. For the total cell count, the suspension was plated on R2A agar (Sigma Aldrich, Diegem, Belgium) with cycloheximide (200 mg/l). Agrobacteria colonies were selected and identified following Shams et al. (2012). *A. rhizogenes* was isolated using 2E-Te containing erythritol and 320 mg/l K_2TeO_3 with cycloheximide. After 5 days of incubation at 28°C, colony forming units (CFU) were counted for both R2A and 2E-TE medium. The calculation of the CFU was following the procedures outlined by Sutton (2011), where the detection limit was equal to 1 CFU at the lowest dilution.

2.6 DNA extraction

Total DNA was extracted using physical disruption with the bead beating method from (Hernandez-Sanabria et al. (2012)). Cells were lysed in a FastPrep-96 homogenizer (MP Biomedicals, Illkirch, France) and DNA was precipitated with cold ethanol and resuspended in 30 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). Concentration and quality of DNA were measured based on the absorbance at 260 and 280 nm in a Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.7 Identification of agrobacterium sp. at strain/biovar level

The potential presence of pathogenic *Agrobacterium* sp. strains was analyzed by multiplex PCR, targeting the 23S rRNA gene (Puławska et al. 2006). Universal forward primer UF and four reverse primers specific for *A. tumefaciens* (biovar 1), *A. rhizogenes* (biovar 2), *A. vitis*, and *A. rubi*, were used. Conditions of the PCR were described elsewhere (Puławska et al. 2006); the primer pairs UF/B1R,UF/B2R, UF/AvR and UF/ArR were employed to amplify fragments of 184, 1066, 478, and 1006 bp length, respectively (Kuzmanović et al. 2012). Pathogenic plasmid detection revealed the presence of the *virC* pathogenicity gene located on the rhizogenic (Ri) plasmid (Suzaki et al. 2004); PCR conditions for detection of the *virC* gene followed Kuzmanović et al. (2012). Additional confirmation was performed in randomly selected wild type isolates; colony PCR was applied using the protocol described above.

2.8 Community PCR-DGGE analysis

PCR amplifications of the V3 region (~200 bp) of the 16S rRNA gene of bacteria were performed with universal bacterial primers as described by Øvreås et al. (1997). PCR products were purified prior to fingerprinting analysis and DGGE was run on 1× TAE buffer (AppliChem, Darmstadt, Germany) with a 6% polyacrylamide gel with a 30 to 50% linear denaturing gradient, using the Bio-Rad DCode universal mutation detection system (BioRad, Hercules, CA, USA). Running conditions and analysis using BioNumerics software, version 5.1 (Applied Maths, Sint-Martens Latem, Belgium) were reported by Hernandez-Sanabria et al. (2012). New band categories including all the detected bacterial phylotypes on the growing media were created. Frequency of phylotypes exclusively present in samples with hairy roots was determined adapting the methodology of Hernandez-Sanabria et al. (2013), for performing Fisher Exact test in R (Team 2012).

2.9 Illumina library generation

The V5-V6 region of the 16S rRNA gene was amplified using reported primers (Bohorquez et al. 2012). Libraries were prepared by pooling equimolar ratios of amplicons (200 ng of each sample), tagged with a unique barcode (Camarinha-Silva et al. 2014). Resulting libraries were sequenced on a MiSeq (Illumina, Hayward, CA, USA) paired and joined, but only forward reads were selected for the final analysis (140 nt). A quality filter program that runs a sliding window of 10% of the read length, and calculates the local average score based on the Phred quality score of the FASTQ file, was used to trim the 3'-ends of the reads that fell below a quality score of 10. Reads with an N character in their sequence, mismatches within the primers and barcodes or more than 8 homopolymers stretches were discarded. Following primer sequences trimming, sequences were separated based on their barcodes. Number of representative phylotypes were generated using the Uclust algorithm on USEARCH (Edgar 2010) by clustering at 97% similarity (1 mismatch), with a confidence level of at least 80, with Cyanobacteria, Eukaryota, and Archaea lineages removed. Filtered database contained only phylotypes present in at least a) one sample at an abundance higher than 1%, b) in 2% of samples at a relative abundance above 0.1%, and 3) in 5% of the samples at any abundance level (Camarinha-Silva et al. 2014). Hence, a total of 475995 reads were obtained. Sequence composition of the dataset was compared using the RDP Classifier tool (Wang et al. 2007) and SILVA database (Pruesse et al. 2007).

After examining read counts, data were randomly rarefied to a chosen maximum depth of 17135 sequences, using the phyloseq package from R (McMurdie and Holmes 2013) and rarefaction curves were plotted using the vegan package in R (Oksanen et al. 2007). Relative abundances of the top twelve taxa, with their deepest possible RDP classification up to the family level were determined and plotted

as bar charts (Kerckhof et al. 2014). If any OTU was not classified up to a family level, the consensus sequence was blasted using the NCBI database and taxonomic classification was obtained. Within each sample, total number of species, Fisher's diversity, Shannon, Simpson and inverse Simpson indices were calculated to assess the alpha diversity. Pielou's index was used as indicator of evenness in the community. Differences in alpha diversity and evenness measures among horticultural growing medium were compared using a repeated measures mixed model in SAS (version 9.3, SAS Institute, Cary, USA), with growing medium type as a fixed effect and comparing multiple means using Tukey test. Hence, the differences in the diversity measures could be attributed to either time point, growing medium type or to the interaction of time*growing medium type. Chao and Bray-Curtis indices were used to construct dissimilarity matrices of the communities. Therefore, beta diversity of the community was determined, and nMDS was employed to visualize the differences among samples, using the vegan package in R (Oksanen et al. 2007). Stratified permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was conducted to explore the percentage of variance that could be explained by the differences in beta diversity. ANOVA was applied to uncover whether one of the growing media was more variable than the other (Oksanen et al. 2007). Differences in relative abundances of bacterial families were compared using a repeated measures mixed model in SAS, with the lsmeans adjustment and Bonferroni correction for multiple comparisons.

2.10 Multivariate statistical analysis

Differences in physicochemical characteristics of each horticultural growing medium were compared using a mixed model in SAS. Pearson correlations were used to determine the interactions between the physicochemical characteristics and significance was assumed at $P < 0.05$. Sixteen variables were included in the analysis (water content, pH(H2O), conductivity, nitrate-N, ammonium-N, phosphorus, potassium, calcium, magnesium, sulphate, sodium, chloride, iron, manganese, CFU of *A. rhizogenes* sp. and total bacteria). Multiple Factor Analysis (MFA) was employed to detect how the relative abundances of families contributed to the differences between growing media across time points. In addition, MFA was applied to the whole set of variables to assess the correlations among the physical, chemical and microbiological variables detected in both types of growing medium. Each group of variables was weighted and results were explained in a factor map (De Tayrac et al. 2009), where the value of the abundance of each bacterial family (vector) for the corresponding growing medium (factor) was plotted. The function MFA from the FactoMineR package (Lê et al. 2008) was performed in R. Bipartite networks were inferred using a pair-wise similarity matrix obtained from the Regularized Canonical Correlation Analysis (González et al. 2012; Lê Cao et al. 2009). The values in the similarity matrix were computed as the correlation between the relative abundances of bacterial families and the growing medium characteristics projected onto the space spanned by the first components retained in the analysis. Three relevant components were obtained setting a threshold of $r \geq 0.5$ and families were disseminated in the plot, in close relation with the variables correlated and with the growing medium where they were more abundant. An additional ordination procedure, Correspondence Analysis (CA), was employed to confirm the relationships among specific bacterial families and the assessed physical and chemical characteristics (Hernandez-Sanabria et al. 2013).

3. Results

3.1 Identification of the microbial community associated with the growing medium

Chitinophagaceae, *Xanthomonadaceae*, *Flavobacteriaceae*, *Hypomicrobiaceae*, *Microbacteriaceae*, *Comamonadaceae*, *Enterobacteriaceae*, *Methylophilaceae*, *Rhizobiaceae*, *Pseudomonadaceae*, and *Sphingobacteriaceae* were the bacterial families with highest relative abundances in both growing media (Figure 2.2A). *Chitinophagaceae*, *Methylophilaceae* and *Hypomicrobiaceae* were abundant in GB, while *Microbacteriaceae* were increased in RW. *Enterobacteriaceae*, *Verrucomicrobiaceae* and *Rhizobiaceae* were abundant in RWS and decreased in GB (Table 2.1 and 2.2). Table 2.1 and 2.2 show the most significant bacterial families, while Figure 2.2A gives an overview of the relative abundances of all the bacterial families present in horticultural growing media. Families with the highest sequence count and their corresponding RDP classification are indicated. RW: mineral growing medium shows the Permutational multivariate analysis of variance (PERMANOVA) confirmed that growing media type, but not time point, significantly contributed to the differences in the relative abundances of bacterial families ($P < 0.05$, Figure 2.2B). Only the most significant bacterial families are presented in Table 2.1., while in Figure 2.2 shows all the bacterial families.

Table 2.1: Effect of time and growing medium type on relative abundance of bacterial families present in horticultural growing media (n = 12). GB, organic growing medium, n = 6. RW, mineral growing medium, n = 6. NS = not significant effect. Different superscripts indicate significantly different means. "0.00" indicates zero relative abundance detected. Only the most significant bacterial families are presented.

Taxonomy	Time point	Growing medium		*P* value	Time effect	Time * growing medium interaction
		GB (Mean % ± SEM)	RW (Mean % ± SEM)			
Acetobacteraceae	**1**	0.18 ± 0.07	0.00	0.03	NS	NS
	2	0.15 ± 0.07	0.00			
	3	0.13 ± 0.07	0.00			
Actinobacteridae	**1**	0.78 ± 0.42	0.07 ± 0.42	0.04	NS	NS
	2	1.81 ± 0.42	0.87 ± 0.42			
	3	1.38 ± 0.42	0.28 ± 0.42			
Chitinophagaceae	**1**	19.47 ± 2.89^{a}	4.42 ± 2.89^{b}	0.05	NS	NS
	2	7.63 ± 2.89	6.65 ± 2.89			
	3	8.16 ± 2.89	6.63 ± 2.89			
Chromatiaceae	**1**	0.10 ± 0.14	0.02 ± 0.14	0.01	NS	NS
	2	0.42 ± 0.14	0.00			
	3	0.80 ± 0.14	0.00			
Conexibacteraceae	**1**	0.30 ± 0.60	0.05 ± 0.60	0.03	0.01	NS
	2	2.11± 0.60	0.10 ± 0.60			
	3	1.92 ± 0.60	0.09 ± 0.60			
Cryomorphaceae	**1**	0.02 ± 0.48	0.84 ± 0.48	0.05	NS	NS
	2	0.02 ± 0.48^{a}	1.63 ± 0.48^{b}			
	3	0.00	0.52 ± 0.48			
Desulfobacteraceae	**1**	0.08 ± 0.09	0.005 ± 0.09	0.0001	0.003	0.003
	2	1.18 ± 0.09^{a}	0.00 ^{b}			
	3	0.62 ± 0.09^{a}	0.00 ^{b}			
Ectothiorhodospiraceae	**1**	0.19 ± 0.08	0.005 ± 0.08	0.05	NS	NS
	2	0.22 ± 0.08	0.02 ± 0.08			

	3	0.13 ± 0.08	0.00			
Erythrobacteraceae	**1**	0.26 ± 0.03[a]	0.05 ± 0.03[b]	0.03	NS	NS
	2	0.10 ± 0.03	0.02 ± 0.03			
	3	0.18 ± 0.03	0.06 ± 0.03			
Gemmatimonadaceae	**1**	1.24 ± 0.19[a]	0.03 ± 0.19[b]	0.0003	NS	NS
	2	1.20 ± 0.19[a]	0.19 ± 0.19[b]			
	3	1.39 ± 0.19[a]	0.03 ± 0.19[b]			
Hahellaceae	**1**	0.08 ± 0.06	0.00	0.04	NS	NS
	2	0.18 ± 0.06	0.00			
	3	0.16 ± 0.06	0.00			
Haliangiaceae	**1**	0.09 ± 0.08	0.005 ± 0.08	0.0002	0.001	0.001
	2	0.41 ± 0.08[a]	0.02 ± 0.08[b]			
	3	1.22 ± 0.08[a]	0.00 [b]			
Hyphomicrobiaceae	**1**	5.69 ± 1.59	1.53 ± 1.59	0.01	NS	NS
	2	8.90 ± 1.59	4.47 ± 1.59			
	3	6.33 ± 1.59	1.76 ± 1.59			
Hyphomonadaceae	**1**	0.46 ± 0.21	0.07 ± 0.21	0.01	NS	NS
	2	0.60 ± 0.21	0.11 ± 0.21			
	3	0.95 ± 0.21[a]	0.04 ± 0.21[b]			
Ignavibacteriaceae	**1**	0.12 ± 0.04	0.00	0.02	NS	NS
	2	0.06 ± 0.04	0.00			
	3	0.17 ± 0.04	0.01 ± 0.04			
Methylocystaceae	**1**	0.14 ± 0.08	0.00	0.03	NS	NS
	2	0.20 ± 0.08	0.00			
	3	0.19 ± 0.08	0.00			
Methylophilaceae	**1**	6.34 ± 1.87	1.55 ± 1.87	0.04	NS	NS
	2	5.97 ± 1.87	4.09 ± 1.87			
	3	7.02 ± 1.87	1.95 ± 1.87			
Microbacteriaceae	**1**	0.28 ± 0.69	1.43 ± 0.69	0.003	NS	NS
	2	0.67 ± 0.69[a]	4.52 ± 0.69[b]			
	3	1.02 ± 0.69[a]	3.94 ± 0.69[b]			
Opitutaceae	**1**	2.98 ± 0.71[a]	0.13 ± 0.71[b]	0.003	NS	NS
	2	4.40 ± 0.71[a]	0.79 ± 0.71[b]			
	3	2.36 ± 0.71	0.14 ± 0.71			
Phyllobacteriaceae	**1**	0.48 ± 0.16	0.005 ± 0.16	0.007	NS	NS
	2	0.76 ± 0.16[a]	0.22 ± 0.16[b]			
	3	0.64 ± 0.16[a]	0.09 ± 0.16[b]			
Prochlorococcaceae	**1**	0.12 ± 0.11	0.07 ± 0.11	0.04	NS	NS
	2	0.11 ± 0.11	0.00			
	3	0.48 ± 0.11[a]	0.00 [b]			
Rhodobacteraceae	**1**	0.41 ± 0.05[a]	0.12 ± 0.05[b]	0.02	0.04	NS
	2	0.18 ± 0.05	0.18 ± 0.05			
	3	0.16 ± 0.05	0.03 ± 0.05			
Rhodocyclaceae	**1**	0.29 ± 0.48	0.16 ± 0.48	0.04	0.02	NS
	2	0.69 ± 0.48	0.30 ± 0.48			

	3	3.22 ± 0.48[a]	0.73 ± 0.48[b]			
Sinobacteraceae	**1**	0.39 ± 0.11	0.02 ± 0.11	0.003	NS	NS
	2	0.46 ± 0.11	0.11 ± 0.11			
	3	0.65± 0.11[a]	0.06 ± 0.11[b]			
Sorangiineae	**1**	1.06 ± 0.63	0.03 ± 0.63	0.0004	0.02	0.02
	2	3.58 ± 0.63 [a]	0.03 ± 0.63[b]			
	3	6.18 ± 0.63[a]	0.005 ± 0.63[b]			
Verrucomicrobiaceae	**1**	1.23 ± 0.40	0.57 ± 0.40	0.02	NS	NS
	2	2.72 ± 0.40 [a]	0.98 ± 0.40[b]			
	3	1.17 ± 0.40	0.52 ± 0.40			
Vibrionaceae	**1**	0.12 ± 0.01	0.16 ± 0.01	NS	0.0002	NS
	2	0.03 ± 0.01	0.00			
	3	0.00	0.00			
Xanthobacteraceae	**1**	0.75 ± 0.33	0.08 ± 0.33	0.02	NS	NS
	2	1.52 ± 0.33 [a]	0.15 ± 0.33[b]			
	3	0.74 ± 0.33	0.09 ± 0.33			

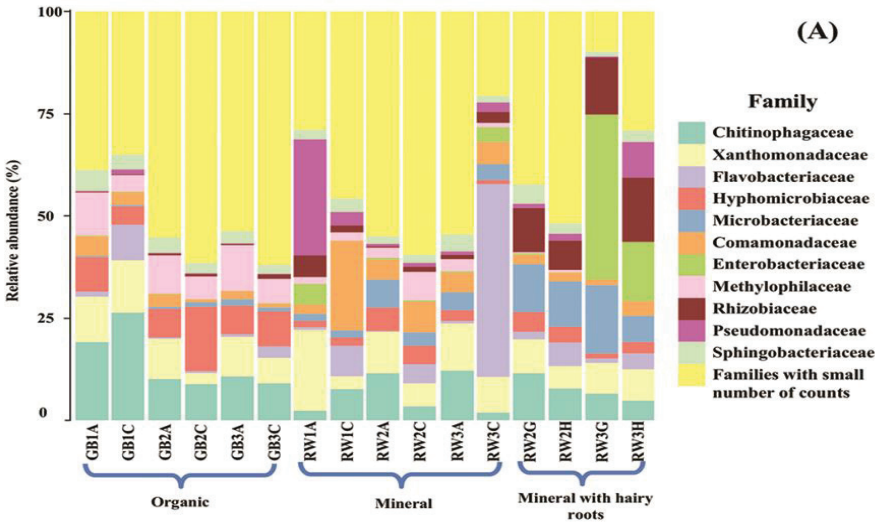


Figure 2.2: (A) Relative abundances of the bacterial families present in horticultural growing media. Families with the highest sequence count and their corresponding RDP classification are indicated. RW: mineral growing medium; GB: organic growing medium, RWS: mineral medium with hairy roots. Dataset was rarefied to the lowest sequence count; relative abundances were calculated summing the counts of OTUs belonging to the same family.

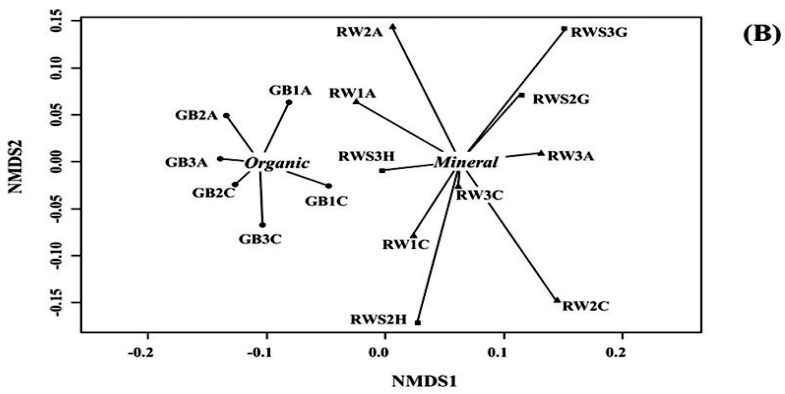


Figure 2.2: (B) Community structure was significantly different between growing media types. Analysis of multivariate homogeneity of group dispersions (variances) was performed and non-metric multidimensional scaling analysis was used to assess the similarity among bacterial communities. Symbols indicate the growing medium type: circles, organic growing medium (GB); triangles, mineral growing medium (RW); squares, mineral growing medium with hairy roots (RWS). The number in the legend specifies the time point and the letter refers to the sample replicate. For instance, "GB1A" refers to the replicate "A" of organic growing medium, collected at the first time point.

Analysis of the DGGE profiles showed that RWS samples grouped together regardless of time, while the rest of the samples tended to cluster according to time point (Supplementary figure 2.1). The total number of species was higher in GB ($P < 0.05$), while diversity and evenness between GB and RW was significantly different across time points ($P < 0.05$, Supplementary table 2.1). RW and RWS showed consistent similarities in their diversity and evenness metrics (Supplementary table 2.2).

Table 2.2: Effect of time and hairy roots presence on relative abundance of bacterial families present in horticultural growing media (n = 10). RW, mineral growing medium, n = 6. RWS, mineral growing medium with hairy roots, n = 4. NS = not significant effect. Different superscripts indicate significantly different means. "0.00"indicates zero relative abundance detected.

Taxonomy	Time point	Growing medium		*P* value	Time effect	Time * hairy roots interaction
		RW (Mean ± SEM)	RWS (Mean ± SEM)			
Hyphomicrobiaceae	**1**	1.53 ± 0.57	0.00	NS	0.01	NS
	2	4.47 ± 0.57	3.86 ± 0.57			
	3	1.76 ± 0.57	1.68 ± 0.57			
Methylophilaceae	**1**	1.54 ± 0.96	0.00	0.03	NS	NS
	2	4.09 ± 0.96 [a]	0.35 ± 0.96 [b]			
	3	1.95 ± 0.96	0.005 ± 0.96			
Planctomycetaceae	**1**	0.005 ± 0.05	0.00	0.0003	0.0007	0.0002
	2	0.02 ± 0.05 [a]	1.06 ± 0.05 [b]			
	3	0.07 ± 0.05	0.01 ± 0.05			
Rhizobiaceae	**1**	2.94 ± 0.85	0.00	0.0001	0.03	0.05
	2	1.02 ± 0.85 [a]	7.91 ± 0.85 [b]			

	3	2.02 ± 0.85 [a]	13.02 ± 0.85 [b]			
	1	0.57 ± 0.45	0.00			
Verrucomicrobiaceae	**2**	0.98 ± 0.45 [a]	3.96 ± 0.45 [b]	0.02	0.02	0.2
	3	0.52 ± 0.45	0.53 ± 0.45			
	1	0.16 ± 0.005	0.00			
Vibrionaceae	2	0.00	0.01 ± 0.005	NS	<0.0001	NS
	3	0.00	0.00			

Multiple Factor Analysis (MFA) showed that families associated with GB were represented in Dimension 1 ($P < 0.0001$, Figure 2.3A), accounting for 28% of the variance in relative abundances among all the samples. *Gemmatimonadaceae, Sinobacteraceae*, *Sorangiineae, Opitutaceae*, *Desulfobacteraceae, Actinobacteridae, Hahellaceae, Gaiellaceae, Hypomicrobiaceae, Methylophilaceae, Acetobacteraceae, Methylocystaceae, Conexibacteriaceae, Xanthobacteraceae* and Unclassified *Nitrospira* were significantly associated with GB. Bacterial families that correlated with RW were represented in Dimension 3 ($P < 0.05$) and explained 11% of the total variance. *Pseudonocardineae, Propionibacterineae, Bacteroidaceae, Commamonadaceae*, Incertae *Rhizobiales* and *Cryomorphaceae* were associated with this dimension. *Rhizobiaceae*, *Verrucomicrobiaceae*, *Planctomycetaceae*, *Simkaniaceae*, *Piscirickettsiaceae*, and *Caldilineaceae* were families associated with RWS and included in Dimension 5 ($P < 0.05$, Supplementary table 2.3).

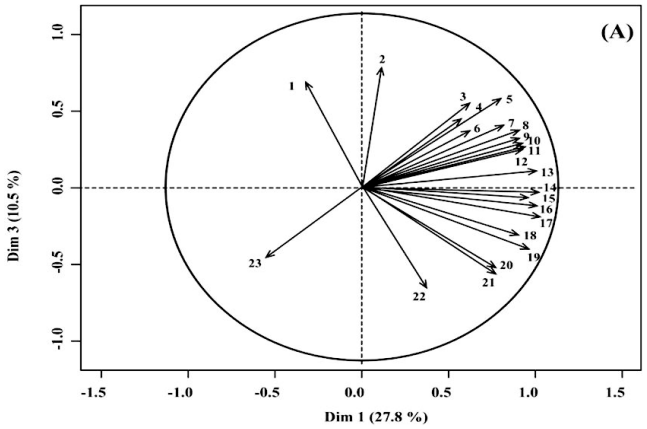


Figure 2.3: (A) Variations in the abundance of bacterial families in horticultural growing media. According to the correlation circle, the families belonging to the first component of the Multiple Factor Analysis (Dimension 1) are negatively correlated to the abundance of species belonging to Rhizobiaceae. Since there are more families in Dim 3, their contribution to the overall variance among samples is smaller. Dimension 3 described the families that were significantly correlated with RW ($P < 0.05$). 1, Propionibacterineae; 2, Pseudonocardineae; 3, Rhodobacteraceae; 4, Caedibacter; 5, Incertae Rhizobiales; 6, Unclassified Nitrospira; 7, Methylophilaceae; 8, Gaiellaceae; 9, Acetobacteraceae; 10, Actinobacteridae; 11, Xanthobacteraceae; 12, Hahellaceae; 13, Sinobacteraceae; 14, Desulfobacteraceae; 15, Hyphomicrobiaceae; 16, Opitutaceae ; 17, Gemmatimonadaceae; 18, Methylocystaceae; 19, Sorangiineae; 20, Hyophomonadaceae; 21, Chromatiaceae; 22, Rhodocyclaceae; 23, Rhizobiaceae.

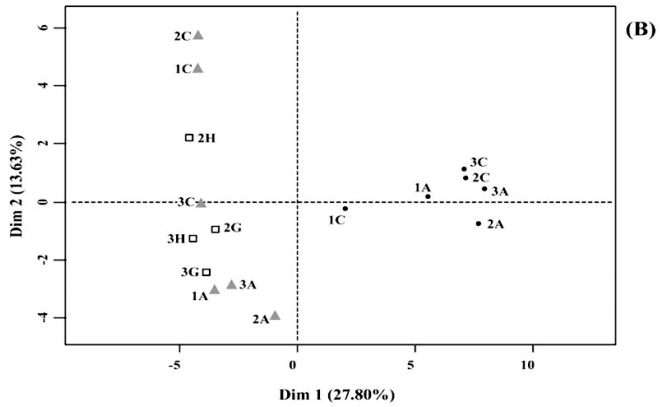


Figure 2.3: (B) Multiple Factor Analysis map indicated that samples from organic growing medium (GB) displayed similar abundances across time points and differed from those in mineral medium (RW). Bacterial family abundances in samples of RW with hairy roots (RWS) were similar to those in RW. Symbols indicate the growing medium type: black circles for GB, grey triangles for RW and white squares for RWS. The number in the legend specifies the time point and the letter refers to the sample replicate. For instance, the circle labelled as "1A" refers to the replicate "A" of GB, collected at the first time point.

Tukey's test for pairwise comparisons of group mean dispersions was performed using the vegan package in R. As demonstrated by the diversity and evenness measures (Supplementary table 2.1 and Supplementary table 2.2), the interaction between time and growing medium type was significant at the third time point ($P < 0.05$). Based on the relative abundances of the bacterial families and on the measures of alpha diversity and evenness, we validated the presence of distinctive and stable microbial communities associated with each growing medium [Table 2.1 and 2.2, Figure 2.3B].

3.2 Physicochemical and biological environments are unique between the different growing media

Plant yield was determined at the end of the growing season for both the mineral and the organic growing medium, and resulted in total accumulated yield (fresh weight) of 59.27 ± 1.52 $kg.m^{-2}$ and 61.59 ± 0.86 $kg.m^{-2}$ respectively. Calcium, magnesium, sulphate, nitrate-N, sodium and conductivity were higher in GB than that in RW ($P < 0.05$), while ammonium-N, potassium, iron, and manganese were significantly higher in RW in comparison with GB ($P< 0.05$, Supplementary table 2.4). Ammonium-N, pH(H2O), conductivity, potassium, sodium, iron and chloride were the highest in RWS ($P < 0.05$, Supplementary table 2.5). Positive correlations between conductivity and nitrate-N were consistently detected in GB, while ammonium-N was associated with total CFU only at the third time point ($P < 0.05$, Supplementary table 2.6). In RW, pH(H_2O) was positively correlated with the *Agrobacterium* sp. CFU, while conductivity, ammonium-N, sulphate and sodium were negatively correlated with the total CFU. Only sulphate was positively correlated with sodium across time points ($P < 0.05$, Supplementary table 2.7). Positive correlations between calcium and *Agrobacterium* sp. and total CFU were found in

RWS at all times ($P < 0.05$, Supplementary table 2.8). In contrast, water content was negatively correlated with *Agrobacterium* sp. and total CFU when the hairy roots were first detected ($P < 0.05$).

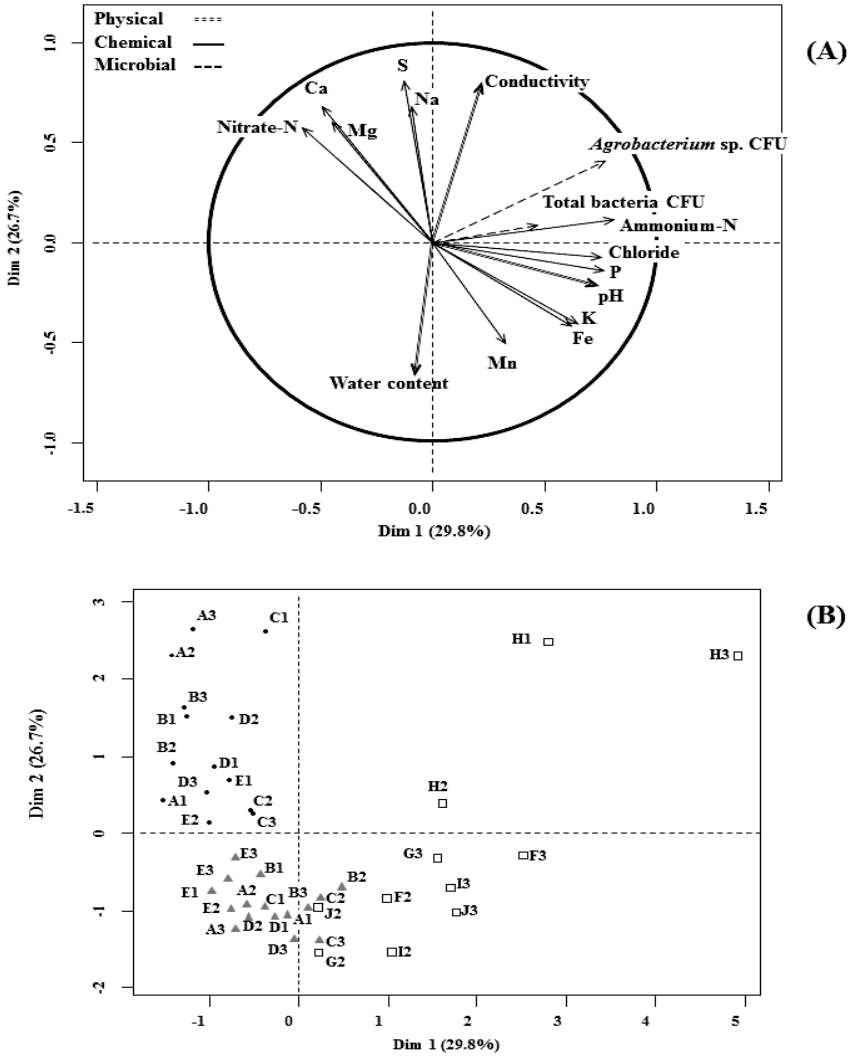


Figure 2.4: Physical and chemical characteristics of the growing media are unique for each environment. (A) Multiple Factor analysis of the physical and chemical characteristics of horticultural growing media. Correlation circle indicates the contribution of the variables driving the differences among growing media. Long vectors in the same direction indicate positive correlations among variables, whereas long vectors in the opposite direction indicate negative ones. (B) Multiple Factor Analysis highlighted the similarities among growing media samples over time, based on their physical and chemical features. Symbols indicate the growing medium type: black circles for GB, grey triangles for RW and white squares for RWS. The number in the legend specifies the time point and the letter refers to the sample replicate. For instance, the circle labelled as "1A" refers to the replicate "A" of GB, collected at the first time point.

The goal of the MFA was twofold: to discriminate growing media based on the measured variables and to uncover the correlations among the physicochemical and biological characteristics within growing medium. In general, MFA showed that total bacteria, *Agrobacterium* sp. CFU, water content, pH(H_2O), sulphate and conductivity were the traits with highest contribution to the total variance among samples (Figure 2.4A). Ammonium-N and *Agrobacterium* sp. CFU were the two variables with the highest correlation to Dimension 1 ($P < 0.0001$, Supplementary table 2.9), which accounted for 29.8% of the variance. The square correlation ratios measure the degree of association between variables and a particular axis. Thus, the cos^2 between the coordinates of the samples and growing medium type revealed that the above were the main characteristics describing the RWS medium on Dimension 1 ($cos^2 > 0.5$). Dimension 2 (26.7% of the total variance) was constructed by the features of GB (sulphate, conductivity, sodium, magnesium, calcium and nitrate-N), while potassium, manganese, iron and water content were included in Dimension 3 with RW ($P < 0.05$, 13.6% of the variance). We confirmed that each growing medium was characterized by a unique set of physicochemical and biological variables, which is preserved over time (Figure 2.4B).

3.3 Correlation of bacterial families with physicochemical and biological characteristics

Instead of computing the Pearson correlation coefficients between each pair of variables, bipartite networks were inferred using a pair-wise similarity matrix obtained from the Regularized Canonical Correlation Analysis (Figure 2.5).

The values in the similarity matrix were computed as the correlation between the relative abundances of bacterial families and the characteristics of the growing medium projected onto the space spanned by the first components retained in the analysis. Three relevant components were obtained setting a threshold to 0.5. In this way, ammonium-N was correlated with *Rhizobiaceae* abundance. Families associated with RW were correlated with iron ($P < 0.05$), while potassium, magnesium, calcium and nitrates were associated with GB ($P < 0.05$). Correspondence Analysis (CA, Supplementary figure 2.2) was used to reveal the association between growing medium and physicochemical variables and relative abundances.

Chi-square statistic indicated strong link between growing medium and both physicochemical variables and relative abundances ($P < 0.05$). The coordinates of row/column variables represent the number of standard deviations the row/column variables are away from the barycenter (Bendixen 1996). Therefore, the highest coordinates in Dimension 1 belonged to *Agrobacterium* sp. CFU, *Solimonadaceae*, Ammonia-N and P (rows) and RWS and time point 3 (columns), all of which explained 92.9% of the variance. Dimension 2 was driven by the relationships among the most abundant families detected in GB at time point 2. The observed interactions confirmed the results of previous correlation analyzes.

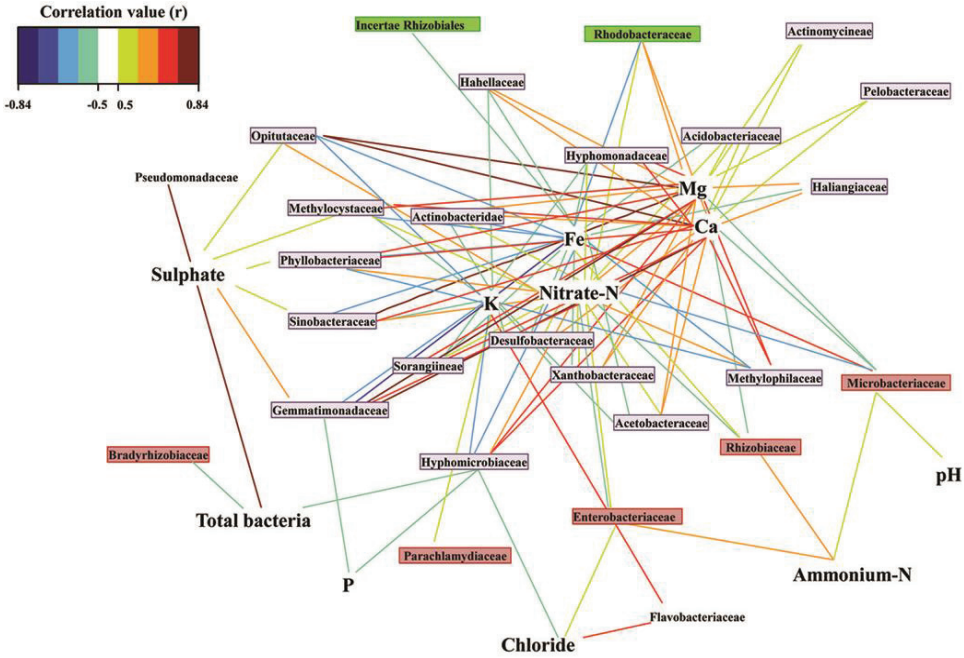


Figure 2.5: Network graph based on the regularized canonical correlations between bacterial family abundance and physicochemical characteristics of growing medium. Correlations (r) have been filtered for an absolute correlation above 0.5 and are colored following the key shown. According to the graphing algorithm, stronger correlations are shorter lines, and families with similar abundances within growing medium tend to cluster closely. This representation reveals the relationship between clusters of families linked to the different physical and chemical characteristics of the environment, thus potentially uncovering growing medium-specific populations. In green, bacterial families correlated with RW; in red, bacterial families associated with RWS, in purple, families correlated with GB.

3.4 The water retention curve of the mineral and organic growing medium

The water retention curves of the two growing media were determined. The measured porosity of the mineral and organic growing medium was 98.34 ± 0.05% and 93.50 ± 0.71% for 10 samples, respectively. The volumetric water content for RWS was 0.98±0.0005 $m^3\ m^{-3}$ at saturation, 0.14±0.008 $m^3\ m^{-3}$ at -1kPa, 0.016±0.002 $m^3\ m^{-3}$ at -5 kPa and 0.01±0.002 $m^3\ m^{-3}$ at -10 kPa. The volumetric water content, on the contrary for GB was 0.93±0.007 $m^3\ m^{-3}$ at saturation, 0.43±0.02 $m^3\ m^{-3}$ at -1kPa, 0.33±0.015 $m^3\ m^{-3}$ at -5 kPa and 0.29±0.007 $m^3\ m^{-3}$ at -10 kPa.

3.5 Potential resistance to external forces differed between growing media

Seven RW samples (46.7% out of fifteen samples) were positive for *A. rhizogenes* biovar 2, whereas samples from GB were negative for any of the tested species of *Agrobacterium* sp. (Supplementary table 2.10). Phytopathogenic *Agrobacterium* sp. strains harbor the genes required for T-DNA process and transfer in the virulence regions (*virC*) of the root inducing (pRi) plasmids (Sawada et al. 1995)·(Zhu et al. 2000). Therefore, RW samples positive for *A. rhizogenes* biovar 2 and all RWS samples were

screened for the presence of the *virC* pathogenicity gene, and all RWS tested positive. Plate counts on the selective medium confirmed the results of the multiplex PCR. Results of the colony PCR in randomly selected wild type isolates validated the presence of the *virC* gene and of *A. rhizogenes* biovar 2 in RWS (Supplementary table 2.7). Differences in *Agrobacterium.* sp. CFU between growing media were influenced by time and were lower in RW when compared with RWS ($P < 0.05$, Supplementary table 2.7).

4. Discussion

We hypothesized that mineral (RW) and organic (GB) growing media in a closed soilless culture system develop a distinctly different community structure with respect to species richness, diversity, evenness and resistance against *Agrobacterium rhizogenes*. Based on the relative abundances of the families associated with each growing medium, we found a significant different microbial community structure in GB, RW and RWS although they are shaped by the same fertigation solution with known pH, EC, and nutrient composition. Moreover, time and growing medium type had a significant effect on species richness, diversity and evenness. GB had a higher species richness, diversity and evenness compared to RW. Further, we identified water content, potassium content, pH(H_2O) and conductivity as the main physicochemical characteristics that are positively correlated with the microbial communities in the growing medium.

4.1 The microbial community associated with the growing medium

High-throughput sequencing combined with molecular techniques uncovered the structure of the growing medium-associated microbiota. GB harbored higher bacterial diversity than RW and RWS. Further, GB displayed similar abundances of bacterial families across time points, while both RW and RWS displayed larger variability. These differences could be associated with the different structure and composition of the two types of growing medium, which may provide unique niches for the microbial community (Sonneveld and Voogt 2009b). This is in agreement with the study of Vallance et al. (2010), who stated that the density and the biodiversity of the microbial community may be affected by the type of growing medium (organic or inorganic), the nutrients in the solutions and the age and cultivar of the plant species. Biodiversity in soilless systems with mineral growing media is low at the start of a crop (Postma 2009), then it increases within weeks (Postma et al. 2000), reaching stability after six weeks of plant growth (Calvo-Bado et al. 2006). As described in previous reports (Sonneveld and Voogt 2009b), our uncultivated RW medium showed low amounts of nutrients and total bacteria CFU ($< 10^2$ CFU g^{-1}) in comparison with GB (2.2×10^7 CFU g^{-1}). Quantification of viable cell counts (CFU) from the culturable aerobic microflora colonizing different parts of the system, such as root zone, nutrient solution, growing media and system devices (tubes, gutters, etc.), has been performed (Strayer 1994). However, only 0.1 to 1% of the microflora may be recovered from techniques based on a plate culturing on R2A agar, providing limited information about the entire community present on each growing medium. For this reason, we complemented cultivation studies with molecular characterization of the microbial communities. From this study it was clear that the microbial community structure was distinctly different between growing media and GB showed lower variability over time compared to RW.

4.2 The microbial community in relation to the plant

The increase in microbial biodiversity observed in the growing medium can be attributed to plant activity. Plants exude up to 21% of their photosynthetically fixed carbon into the root-soil interface (Marschner 2011) feeding the microbial communities and influencing their activity and diversity (Mendes et al. 2011). Berendsen et al. (2012) suggested that plant species can select bacteria through the production of specific root exudates and hence shape the microbiome of the plant. We used eggplants grafted on a tomato root stock known for its high exudation capacity (*Solanum lycopersicum* L. x *Solanum habrochaites*). It is a well known fact that root exudation changes with plant age (Rovira and Harris 1961), hence changes in microbial community structures are most likely caused by changes in root exudation patterns (Marschner et al. 2002; Micallef et al. 2009; Rosberg 2014). As plants were of the same age, showed comparable growth characteristics and yield, the root exudation in both growing media was estimated to be similar. We found that even after six months, the microbial community in the mineral growing medium (both in RW and RWS) showed high variability across time points. Garbeva et al. (2004) hypothesized that in a stable system, each microhabitat is occupied by organisms capable of colonizing niches. A diverse and stable ecosystem at the microhabitat level will resist environmental stresses (Loreau et al. 2001) and potentially, pathogen invasion. Mendes et al. (2011) suggested that the relative abundance of several bacterial taxa may be an indicator of disease suppression. In this way, increased resistance to pathogen invasion may be related to the total microbial biomass in the growing medium, which competes with pathogens for resources or may cause inhibition through direct antagonism (Weller et al. 2002). Mendes et al. (2011) identified Actinobacteria, γ- and β-Proteobacteria (*Pseudomonadaceae*, *Burkholderiaceae*, *Xanthomonadales*) and Firmicutes (*Lactobacillaceae*) as the most dynamic taxa associated with disease suppression in natural soil. In our study, *Rhodocyclaceae* and *Methylophilaceae* (β-Proteobacteria), were correlated to GB, as well as other α-, β and ϒ-Proteobacteria, such as *Hyphomicrobiaceae*, *Xanthobacteraceae*, *Phyllobacteriaceae*, and *Chromatiaceae*. Actinobacteria such as *Gaiellaceae* and *Conexibacteraceae* were also positively correlated with GB. Furthermore, the abundance of *Rhizobiaceae* (such as *Agrobacterium* sp.) was negatively correlated to the abundances of *Methylophilaceae* and *Saprospiraceae* in GB. Thus, the relative abundance of several taxa and the stability of a microbial community may be related to the resistance against external invaders, supporting the theory of general suppression. Even though *Agrobacterium* sp. was detected in both growing media (on average 7.6×10^3 CFU mL^{-1} in GB, 2.4×10^4 in RW and 1.0×10^6 CFU mL^{-1} in RWS, across time points), samples from GB were negative for the presence of the *virC* pathogenicity gene. Neither the total CFU nor the presence of particular microbial taxa have been directly associated with resistance to *Agrobacterium rhizogenes* (Berendsen et al. 2012).

4.3 The microbial community in relation to the physico-chemical characteristics

The plant, as well as the complex biological, chemical and physical interactions in the growing medium influence the microbial communities of the rhizosphere. Indeed, according to Khalil and Alsanius (2001), the differences in microbial community composition between new, sterile and reused mineral growing media were most likely caused due to the deviating chemical and physical properties of the two growing media, indicating the growing media itself has an influence on the microbial community composition. The mineral and the organic growing medium show differences in the form of the water retention curve. This may be explained by differences in the bulk solid phase of the two different growing media. The mineral growing medium has a lower dry bulk density and a higher water content at saturation. The water content of the organic growing medium at -1kPa, -5kPa and -10 kPa is higher compared to the mineral growing medium. The water retention curve of the used mineral growing

medium was comparable with what has been reported earlier in literature (Wallach 2008). Water is a highly variable component in soilless culture systems and is an important factor affecting the growth of microorganisms (Madigan et al. 2008) and the microbial community structure. In accordance with Da Silva et al. (1994), the mineral growing medium in this study was characterized by a very high water content at saturation (h = 0), which decreased sharply with decreasing h, approaching zero at -5 kPa (Da Silva et al. 1993; De Swaef 2011; Ilstedt et al. 2000; Jones 2013). As a result, microorganisms become more nutrient-limited in RW because nutrient diffusion is even more restricted (Stark and Firestone 1995). The microbial community associated with RW will also experience higher decreases in osmotic potential as RW dries faster than GB. Both, low matric and low osmotic potential, have a negative effect on microbial activity and change community composition. The study of Chowdhury et al. (2011a) showed that low matric potential has a stronger negative effect than low osmotic potential. Therefore, substrate diffusion and cell motility at low matric potential is more detrimental to microorganisms in RW compared to GB than low osmotic potential at optimal water content. These differences in water retention might explain the high variability of the microbial community associated with RW over time compared to GB.

Previous reports identified pH as the main driver of microbial communities in soil (Lauber et al. 2009). In addition, the study of Dumbrell et al. (2010) showed that a community that although influenced by chance driven processes, still responded in a predictable manner to a major abiotic niche axis, such as soil pH. Water content is often associated with pH(H_2O) and may have impacted the community composition among GB, RW and RWS. Moreover, GB showed higher relative abundances of *Actinobacteridae* and α-Proteobacteria, which have been associated with soil acidity (Lauber et al. 2009). These results indicate that the physico-chemical characteristics of the organic growing medium support the presence of a stable microbial community.

Calcium, magnesium, sulphate, nitrate-N, sodium and conductivity were positively correlated to GB and no hairy roots syndrome occurred in combination with GB. There is a clear indication that the physico-chemical environment is an important factor whether biocontrol organisms can inhibit the crazy roots causing pathogen. As indicated by Bosmans et al. (2016a), the nutrient composition of the agar affects *in vitro* screening of biocontrol activity of antagonistic microorganisms. A non-metric multidimensional scaling (nMDS) plot showed that Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} en Zn^{2+} en Al^{3+} separated the agars in two groups, with one group showing antagonistic activities and another not showing antagonistic activity. For the particular antagonistic interactions investigated, mentioned study suggests an important role of Ca^{2+}, which is in agreement with our results, to produce and/or secrete potential toxins/antibiotics against rhizogenic *Agrobacterium*. Whereas the importance of Ca^{2+} as a cell regulator is well established in eukaryotes (Bode et al. 2002), little is known about the precise role of Ca^{2+} in prokaryotes. Nevertheless, recent research suggests the possibility that, as in eukaryotes, Ca^{2+} plays a role in signal transduction in bacteria modulating specific functions or generating a specific response (Dominguez 2004). The exact function of Ca^{2+} in the antagonistic interaction investigated here remains, however, to be unraveled.

High yields of hairy roots indicating *A. rhizogenes* invasion have been observed when the nitrate-ammonium (NO_3^--N/NH_4^+) ratio was close to 5 with 115 mg NH_4^+-N L^{-1} of soil and 553 mg NO_3-N L^{-1} of soil (Bensaddek et al. 2001). In our test, the NO_3^--N/NH_4^+ -N ratio was 2.3 for RWS, 31.9 for RW and 147.3 for GB. The low ammonia concentration and the low pH(H_2O) in the GB medium may explain the absence of hairy roots and potentially shaped the microbial community composition. Ammonium can

also be produced under environmental stress (Bittsánszky et al. 2015). Plants infected by *Agrobacterium tumefaciens* and *A. rhizogenes* express the ammonium-producing enzyme ornithine cyclodeaminase (Sharma et al. 2009) and *Pseudomonas syringae* pv. *tabaci* produces tabtoxinine-β-lactam (a potent inhibitor of glutamine synthetase) in infected tissues, leading to the accumulation of phytotoxic concentrations of ammonium (Ito et al. 2014). The necrotrophic tomato (*Solanum lycopersicon*) pathogen *Colletotrichum coccodes* secretes ammonium into the fruit tissues during ripening, and induces host programmed cell death (Alkan et al. 2012).

Our methodology provided an overview into the complex physico-chemical and biological interactions in horticultural media. These results support our hypothesis that there are fundamental differences in microbial community composition, structure and variability over time between the bacterial communities associated with each type of horticultural growing medium. Moreover, these differences could be attributed to differences in physico-chemical characteristics. Diverse and competitive microbial communities may provide different and unique functionalities. As a consequence, the bacterial community inhabiting the GB medium may have provided temporal stability and resilience to this heterogeneous and fluctuating environment. Ultimately, the interactions in the resident community may also play a role in the resistance to external forces, such as invasive species competing in conventional soilless culture systems. Future alternative control strategies may involve the evaluation of the suppressiveness of microbial groups and the transfer of suppressiveness to conducive soils with 1–10% suppressive soil (Weller et al. 2002). The described relationships will also contribute to the understanding of the microbial ecology associated with the growing media and the interaction between microbial community and plants. Knowledge regarding these relationships could potentially be used to develop sustainable strategies to increase plant productivity and quality.

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6. Author contributions

Conceived and designed the experiments: OG, EH-S, MCVL, DR, and NB. Performed the experiments: OG, EH-S. Illumina libraries processing: RJ, RV-V. Data mining, statistical analysis, results interpretation, figure and table preparation: EH-S, OG. Contributed reagents/materials/analysis tools: NB, MP, and DHP. Wrote the paper: OG, EH-S.

Supplementary information

Supplementary table 2.1: Effect of time and growing medium type on species richness (total species), diversity (Shannon, Fisher's alpha, Simpson and Inverse Simpson indices), and evenness (Pielou's index) of horticultural growing media (n = 12). RW, mineral growing medium, n = 6. GB, organic growing medium, n = 6. NS = not significant effect. Different superscripts indicate significantly different means.

Index	Time point	Growing medium		*P* value	Time effect	Time * growing medium type interaction
		GB (Mean ± SEM)	RW (Mean ± SEM)			
Total species	**1**	209.5 ± 15.48	169 ± 15.48	0.01	NS	NS
	2	222 ± 15.48	182.5 ± 15.48			
	3	235 ± 15.48 [a]	177 ± 15.48 [b]			
Shannon	**1**	4.36 ± 0.25 [a]	3.46 ± 0.25[b]	0.003	NS	NS
	2	4.64 ± 0.25 [a]	3.95 ± 0.25[b]			
	3	4.62 ± 0.25[a]	3.24 ± 0.25[b]			
Fisher	**1**	34.92 ± 2.78	27.16 ± 2.78	0.006	NS	NS
	2	37.92 ± 2.78	29.37 ± 2.78			
	3	40.35 ± 2.78 [a]	27.89 ± 2.78[b]			
Simpson	**1**	0.98 ± 0.03	0.92 ± 0.03	0.04	NS	NS
	2	0.98 ± 0.03	0.96 ± 0.03			
	3	0.98 ± 0.03[a]	0.86 ± 0.03[b]			
Inverse Simpson	**1**	40.03 ± 4.50 [a]	14.10 ± 4.50 [b]	<0.0001	0.03	NS
	2	62.27 ± 4.50 [a]	25.46 ± 4.50 [b]			
	3	57.54 ± 4.50 [a]	9.26 ± 4.50 [b]			
Pielou	**1**	0.82 ± 0.04[a]	0.68 ± 0.04[b]	0.003	NS	NS
	2	0.86 ± 0.04	0.76 ± 0.04			
	3	0.85 ± 0.04[a]	0.63 ± 0.04[b]			

Supplementary table 2.2: Effect of time and hairy roots presence on species richness (total species), diversity (Shannon, Fisher's alpha, Simpson and Inverse Simpson indices), and evenness (Pielou's index) of horticultural growing media (n = 10). RW, mineral growing medium, n = 6. RWS, mineral growing medium with hairy roots, n = 4. NS = not significant effect. Different superscripts indicate significantly different means.

Index	Time point	Growing medium		*P* value	Time effect	Time * hairy roots interaction
		RW (Mean ± SEM)	RWS (Mean ± SEM)			
Total species	**1**	169 ± 17.39	-	NS	NS	NS
	2	182.5 ± 17.39	172 ± 17.39			
	3	177 ± 17.39	137.5 ± 17.39			
Shannon	**1**	3.46 ± 0.33	-	NS	NS	NS
	2	3.95 ± 0.33	4.16 ± 0.33			
	3	3.24 ± 0.33	3.16 ± 0.33			
Fisher	**1**	27.16 ± 3.14	-	NS	NS	NS
	2	29.37 ± 3.14	27.42 ± 3.14			
	3	27.89 ± 3.14	21.02 ± 3.14			
Simpson	**1**	0.92 ± 0.04	-	NS	NS	NS
	2	0.96 ± 0.04	0.98 ± 0.04			
	3	0.86 ± 0.04	0.89 ± 0.04			
Inverse Simpson	**1**	14.10 ± 4.51	-	NS	0.01	NS
	2	25.46 ± 4.51	40.20 ± 4.51			
	3	9.26 ± 4.51	12.06 ± 4.51			
Pielou	**1**	0.68 ± 0.05	-	NS	NS	NS
	2	0.76 ± 0.05	0.81 ± 0.05			
	3	0.62 ± 0.05	0.64 ± 0.05			

Supplementary table 2.3: Correlations between relative bacterial abundances and growing medium across time points, indicated by the Multiple Factor Analysis. Dimensions of the MFA can be described by the categorical variables included in the analysis. For each categorical variable (growing medium and time point), a one-way analysis of variance was performed with the coordinates of the samples on the axis, explained by the time point or growing medium type. Then, for each level of the category (i.e. time point 1, time point 2 or time point 3 or growing medium GB), a Hotelling T^2-test was used to compare the average of the category with the general average (using the constraint P i αi = 0, αi = 0). For instance, the coordinates of the relative abundance of family "x" at GB at time point 1 were compared with the average coordinates of the relative abundance of family "x" in GB. The P value associated to this test is transformed to a normal quantile to assess whether the mean of the category is significantly less or greater than 0. Negative values indicate negative correlations.

Dimension	Variance	Descriptor	Estimate (R^2)	P value	Taxon	Correlation	*P* value
					Gemmatimonadaceae	0.903	< 0.0001
					Sinobacteraceae	0.895	< 0.0001
					Sorangiineae	0.852	< 0.0001
					Opitutaceae	0.852	< 0.0001
		Medium	0.909	< 0.0001	*Desulfobacteraceae*	0.837	0.00005
		GB	6.455	< 0.0001	*Actinobacteridae*	0.832	0.00006
		RWS	-3.568	0.06	*Hahellaceae*	0.820	0.00010
		RW	-2.888	0.04	*Xanthobacteraceae*	0.818	0.00011
					Gaiellaceae	0.814	0.0001
					Oceanospirillaceae	0.805	0.0002
1	27.8%				*Hyphomicrobiaceae*	0.803	0.0002
					Methylophilaceae	0.801	0.0002
					Phyllobacteriaceae	0.801	0.0002
					Acetobacteraceae	0.801	0.0002
					Methylocystaceae	0.793	0.0002
					Ignavibacteriaceae	0.787	0.0003
					Haliangiaceae	0.780	0.0004
					Chromatiaceae	0.761	0.0006
					Pelobacteraceae	0.760	0.0006
					Conexibacteraceae	0.759	0.0007

		Hyphomonadaceae	0.730	0.001
		Coxiellaceae	0.725	0.001
		Actinomycineae	0.719	0.002
		Ectothiorhodospiraceae	0.718	0.002
		Incertae Rhizobiales	0.712	0.002
		Unclassified Parcubacteria	0.699	0.003
		Acidobacteriaceae	0.677	0.004
		Prochlorococcaceae	0.674	0.004
		Erythrobacteraceae	0.655	0.006
		Geobacteraceae	0.604	0.01
		Rhodobacteraceae	0.570	0.02
		Unclassified Nitrospira	0.549	0.03
		Caedibacter	0.510	0.04
		Micrococcineae	-0.498	0.05
		Rhizobiaceae	-0.499	0.05
		Microbacteriaceae	-0.577	0.02
2	13.1%	*Incertae Alteromonadales*	0.734	0.001
		Cytophagaceae	0.731	0.001
		Micrococcineae	0.727	0.001
		Family XVII Incertae Sedis	0.721	0.002
		Solimonadaceae	0.721	0.002
		Pseudonocardiaceae	0.705	0.002
		Uncultured Chlorobiales	0.698	0.003
		Acidimicrobineae	0.668	0.005
		Enhygromyxa	0.660	0.005
		Saprospiraceae	0.633	0.008
		Parachlamydiaceae	0.615	0.01
		Bdellovibrionaceae	0.607	0.01
		Kordiimonadaceae	0.537	0.03

					Mycobacteriaceae	-0.544	0.03
					Unclassified Saccharibacteria	-0.577	0.02
					Peptococcaceae	-0.662	0.005
					Xanthomonadaceae	-0.685	0.003
					Pseudonocardineae	0.687	0.003
		Time	0.356	0.06	*Propionibacterineae*	0.606	0.01
		Medium	0.225	0.19	*Bacteroidaceae*	0.604	0.01
		RW	1.815	0.12	*Comamonadaceae*	0.543	0.03
3	10.5%	Tpt 1	1.647	0.18	*Incertae.Rhizobiales*	0.514	0.04
		Tpt 2	0.839	0.31	*Cryomorphaceae*	0.502	0.05
		RWS	-1.852	0.12	*Geobacteraceae*	-0.528	0.04
		Tpt 3	-2.486	0.02	*Rhodocyclaceae*	-0.582	0.02
					Streptomycetaceae	-0.638	0.008
					Gallionellaceae	0.760	0.0006
					Nitrosomonadaceae	0.671	0.004
					Cryomorphaceae	0.620	0.01
		Medium	0.201	0.23	*Propionibacterineae*	0.566	0.02
4	9.2%	RW	1.609	0.14	*Unclassified Saccharibacteria*	0.542	0.03
		RWS	-1.633	0.15	*Solirubrobacteraceae*	0.536	0.03
					Unclassified Microgenomates	0.513	0.04
					Unclassified Nitrospira	-0.516	0.04
					Caedibacter	-0.532	0.03
					Verrucomicrobiaceae	0.653	0.006
					Planctomycetaceae	0.620	0.01
		Time	0.717	0.0003	*Bradyrhizobiaceae*	0.604	0.01
5	7.9%	Medium	0.229	0.18	*Simkaniaceae*	0.576	0.02
		Tpt 2	2.968	0.0003	*Piscirickettsiaceae*	0.562	0.02
		RWS	1.904	0.06	*Caldilineaceae*	0.560	0.02
		Tpt 3	-0.411	0.42	*Solirubrobacteraceae*	0.558	0.02

RW	-1.176	0.30	*Nocardioidaceae*	0.532	0.03
Tpt 1	-2.558	0.01	*Bacteroidaceae*	0.520	0.04
			Vibrionaceae	-0.555	0.03

*Supplementary table 2.4: Effect of time on physical and chemical characteristics of organic and mineral growing media for cultivating eggplants, without hairy roots (n = 30). RW, mineral growing medium (n = 15). GB, organic growing medium (n = 15). NS= no significant effect. * Chemical analysis are expressed as mg L^{-1} growing medium*

Variable	Growing medium		*P* value	Time effect	Time*Growing medium interaction
	RW (Mean ± SEM)	GB (Mean ± SEM)			
Gravimetric water content (kg kg^{-1})	0.8531 ± 0.0103	0.8193 ± 0.0103	0.05	NS	NS
pH(H_2O)	6.58 ± 0.15	6.36 ± 0.15	NS	<0.0001	0.02
Conductivity (µS cm^{-1})	545.87 ± 73.38	845.47 ± 73.38	0.02	NS	NS
Nitrate-N (mg L^{-1*})	263.29 ± 43.63	458.18 ± 43.63	0.01	0.009	NS
Ammonium-N (mg N L^{-1})	8.24 ± 0.75	3.11 ± 0.75	0.001	0.01	NS
P (mg L^{-1})	86.97 ± 17.87	33.79 ± 17.87	NS	0.05	NS
K (mg L^{-1})	315.15 ± 23.24	100.50 ± 23.24	0.0002	NS	0.03
Ca (mg L^{-1})	276.17 ± 76.00	1 558.17 ± 76.00	<0.0001	NS	NS
Mg (mg L^{-1})	73.17 ± 25.47	524.33 ± 25.47	<0.0001	NS	NS
Sulphate (mg L^{-1})	248.53 ± 67.50	737.80 ± 67.50	0.0009	NS	NS
Na (mg L^{-1})	56.17 ± 7.82	147.17 ± 7.82	<0.0001	0.05	NS
Chloride (mg L^{-1})	10.63 ± 0.94	9.62 ± 0.94	NS	NS	0.0002
Fe (mg L^{-1})	4.45 ± 0.32	1.58 ± 0.32	0.0002	0.002	NS
Mn (mg L^{-1})	2.63 ± 0.45	1.46 ± 0.45	0.02	NS	NS
Agrobacterium sp. (CFU mL^{-1})	29 473 ± 11 514	7 618 ± 11 514	NS	NS	NS
Total bacteria (CFU mL^{-1})	10 682 474 ± 2 609 243	6 722 636 ± 2 609 243	NS	0.0004	NS

*Supplementary table 2.5: Effect of time on physical and chemical characteristics of mineral growing medium with and without hairy roots (n = 25). RW, mineral medium without hairy roots (n = 15). RWS, mineral medium with hairy roots (n = 10). NS= no significant effect. * Chemical analysis are expressed as mg L^{-1} growing medium*

Variable	Mineral growing medium		*P* value	Time effect	Time*Hairy roots interaction
	No hairy roots (Mean ± SEM)	Hairy roots (Mean ± SEM)			
Gravimetric water content (kg kg^{-1})	0.8531 ± 0.0093	0.8312 ± 0.0111	NS	NS	NS
pH(H_2O)	6.58 ± 0.19	7.43 ± 0.24	0.02	0.008	NS
Conductivity (µS cm^{-1})	545.87 ± 71.72	815.00 ± 74.65	0.03	NS	0.0003
Nitrate-N (mg L^{-1*})	263.29 ± 17.67	219.04 ± 38.62	NS	0.0006	0.01
Ammonium-N (mg N L^{-1})	8.24 ± 0.70	95.70 ± 1.56	<0.0001	0.002	0.001
P (mg L^{-1})	86.97± 26.28	128.62 ± 32.80	NS	NS	0.04
K (mg L^{-1})	315.15 ± 32.97	541.48 ± 72.87	0.04	NS	NS
Ca (mg L^{-1})	276.17 ± 67.41	174.13 ± 79.80	NS	NS	0.05
Mg (mg L^{-1})	73.17 ± 11.34	94.74 ± 14.54	NS	NS	NS
Sulphate (mg L^{-1})	248.53 ± 47.11	339.13 ± 56.57	NS	0.005	0.03
Na (mg L^{-1})	56.17 ± 7.22	129.63 ± 15.28	0.0009	NS	NS
Chloride (mg L^{-1})	10.63 ± 3.68	33.64 ± 4.51	0.003	NS	NS
Fe (mg L^{-1})	4.45 ± 0.57	7.50 ± 0.79	0.01	NS	NS
Mn (mg L^{-1})	2.63 ± 0.59	2.98 ± 0.71	NS	NS	NS
Agrobacterium sp. (CFU mL^{-1})	29 473 ± 271 685	1 456 074 ± 341 008	0.01	0.02	0.01
Total bacteria (CFU mL^{-1})	10 682 474 ± 4 635 147	10 568 506 ± 6 170 457	NS	NS	0.005

*Supplementary table 2.6: Correlations (r) among physical and chemical characteristics in organic growing medium for cultivating eggplants (GB, n = 15). In parenthesis, the time point when the correlation was observed. ***P < 0.0001, **P < 0.05.*

	pH(H2O)	Nitrate-N	P	Ca	Mg	Sulphate	Chloride	Fe	Mn	Total CFU
Gravimetric water content	-0.944** (2) 0.940** (3)						-0.952**(2)			
pH(H2O)						-0.881** (3)				
Conductivity		0.880** (1) 0.996** (2) 0.989** (3)		-0.876** (3)		0.961** (2) 0.983** (3)				
Nitrate-N			0.978** (2)	-0.886** (3)		0.967** (2) 0.950** (3)				
Ammonium-N	-0.883** (1)	-0.897** (2)				-0.960** (2)				0.883** (3)
P								-0.889** (1)	0.961** (1) 0.975** (2)	
K					-0.953** (2)			-0.945** (2)		
Ca	0.956** (3)									0.893** (1)
Mg	0.920** (3)		-0.929** (1)	0.883** (2) 0.967** (3)				0.938** (2)		
Na					0.938** (3)			0.954** (2)		
Chloride	0.999*** (2)							0.962** (3)		
***Agrobacterium* sp. CFU**										0.959** (3)

*Supplementary table 2.7: Correlations (r) among physical and chemical characteristics in mineral growing medium for cultivating eggplants (RW, n = 15). In parenthesis, the time point when the correlation was observed. ***P < 0.0001, **P < 0.05.*

	pH(H2O)	Conductivity	Nitrate-N	Ammonium-N	P	K	Ca	Mg	Sulphate	Na
Nitrate-N		0.915** (1)								
P	0.933** (3)	0.894** (2)	0.984** (2)	-0.954** (1)						
K	0.930** (3)	0.921** (2)			0.922** (3)					
Ca	0.894** (1)	0.897** (2)	0.951** (2)	-0.941** (1)	0.998** (1) 0.894** (3)					
Mg	0.939** (1) 0.902** (3)	0.906** (2)		-0.912** (1)	0.912** (1) 0.958** (3)	0.907** (3)	0.925** (1) 0.972** (2)			
Sulphate		0.965** (2)	0.967** (1)			0.921** (2)				
Na		0.955** (2)	0.961** (1)		0.932** (2)		0.934** (2) 0.887** (3)	0.955** (2)	0.996** (1) 0.906** (2) 0.941** (3)	
Fe	0.882** (1)						0.883** (3)	0.906** (2)		0.892** (2)
Mn	0.906** (3)	0.970** (2)	0.922** (2)	-0.884** (2)	0.950** (2)	0.880** (2)	0.955** (2)	0.926** (2)	0.874** (2)	0.927** (2)
***Agrobacterium* sp. CFU**	0.891** (2)	-0.945** (1)								
Total CFU		-0.997** (1)		-0.906** (3)					-0.947** (1)	-0.942** (1)

*Supplementary table 2.8: Correlations (r) among physical and chemical characteristics in mineral growing medium for cultivating eggplants, with hairy roots syndrome (RWS, n = 10). In parenthesis, the time point when the correlation was observed. ***P < 0.0001, **P < 0.05.*

	Gravimetric water content	pH(H2O)	Conductivity	Ammonium-N	P	Ca	Mg	Na	*Agrobacterium* sp. CFU
Conductivity		0.949** (3)							
Nitrogen-N		-0.907** (2)							
Ammonium-N		0.876** (2)							
P		0.964** (3)	0.960** (3)						0.995** (2)
K	0.887** (3)			-0.899** (2)	0.893** (2)				
Ca	-0.952** (2)				0.958** (2)				0.953** (2)
Mg	-0.905** (2)				0.953** (2)	0.990** (2)		0.998*** (2)	0.934** (2)
Sulphate		0.913** (3)	0.912** (2)			0.926** (3)			0.974** (3)
Na	-0.918** (2)				0.955** (2)	0.993** (2)			0.939** (2)
Chloride			0.901** (2)						
Fe		-0.907** (2)		-0.898** (2)					
***Agrobacterium* sp. CFU**	-0.883** (2)				0.995** (2)	0.953** (2) 0.963** (3)	0.934** (2)	0.939** (2)	
Total CFU	-0.907** (2)				0.983** (2)	0.991** (2) 0.915** (3)	0.991** (2)	0.994** (2)	0.971** (2)

Supplementary table 2.9: Correlations between physical and chemical characteristics and growing medium across time points, indicated by the Multiple Factor Analysis. Dimensions of the MFA can be described by the categorical variables included in the analysis. For each categorical variable (growing medium and time point), a one-way analysis of variance was performed with the coordinates of the samples on the axis, explained by the time point or growing medium type. Then, for each level of the category (i.e. time point 1, time point 2 or time point 3 or growing medium GB), a Hotelling T^2-test was used to compare the average of the category with the general average (using the constraint P i αi = 0, αi = 0). For instance, the coordinates of calcium in GB at time point 1 were compared with the average coordinates of calcium in GB. The P value associated to this test is transformed to a normal quantile to assess whether the mean of the category is significantly less or greater than 0. Negative values indicate negative correlations.

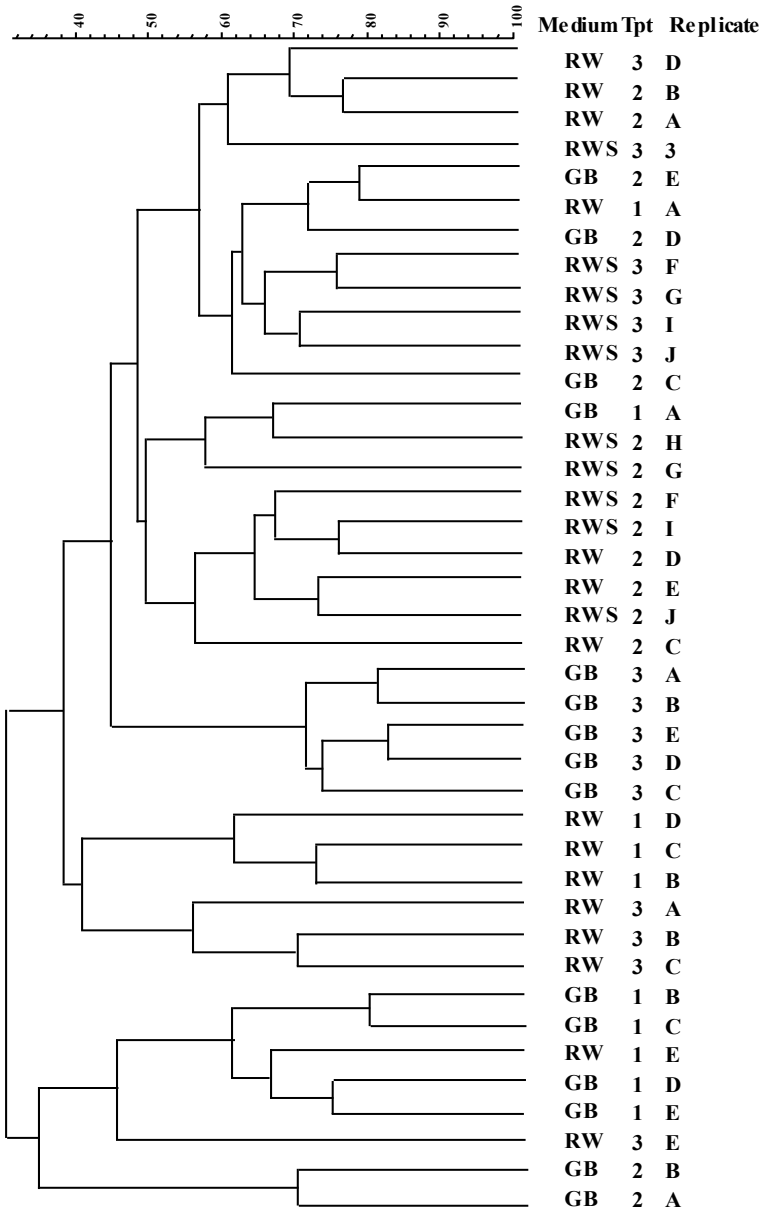
Dimension	Variance	Descriptor	Estimate (R^2)	*P* value	Variable	Correlation	*P* value
1	29.8%	Medium	0.692	< 0.0001	Ammonia-N	0.812	< 0.0001
		Time	0.108	< 0.0001	*Agrobacterium* sp. CFU	0.774	< 0.0001
		RWS	1.590	< 0.0001	Phosphorous	0.772	< 0.0001
		Tpt 3	0.510	0.116	Chloride	0.756	< 0.0001
		RW	-0.464	0.265	pH(H2O)	0.748	< 0.0001
		Tpt 1	-0.618	0.056	Potassium	0.655	< 0.0001
		GB	-1.126	0.000	Iron	0.622	< 0.0001
					Total bacteria CFU	0.476	0.002
					Manganese	0.330	0.04
					Calcium	-0.451	0.003
					Magnesium	-0.473	0.002
					Nitrate-N	-0.589	0.00005
2	26.7%	Medium	0.483	< 0.0001	Sulphate	0.818	< 0.0001
		GB	1.08	< 0.0001	Conductivity	0.806	< 0.0001
		RW	-0.93	< 0.0001	Sodium	0.685	< 0.0001
					Magnesium	0.631	< 0.0001
					Calcium	0.607	0.00003
					Nitrate-N	0.578	0.00008
					Agrobacterium sp. CFU	0.413	0.007
					Potassium	-0.406	0.008

					Iron	-0.419	0.006
					Manganese	-0.507	0.0007
					Gravimetric water content	-0.673	< 0.0001
3	13.6%	Time	0.475	< 0.0001	pH(H2O)	0.429	0.005
		Medium	0.064	0.286	Total bacteria CFU	-0.801	< 0.0001
		Tpt 2	0.609	0.005			
		Tpt 3	0.319	0.308			
		RWS	0.193	0.367			
		GB	0.119	0.464			
		RW	-0.312	0.114			
		Tpt 1	-0.928	< 0.0001			
4	10.8%	Tpt 1	0.141	0.475781	Gravimetric water content	0.661	< 0.0001
					Conductivity	0.396	0.01
					pH(H2O)	-0.337	0.03
5	5.9%	Medium	0.184	0.021	Magnesium	0.410	0.008
		Time	0.041	0.456	Calcium	0.397	0.01
		GB	0.253	0.031	Sulphate	0.317	0.04
		Tpt 3	0.131	0.271	*Agrobacterium* sp. CFU	-0.393	0.01
		Tpt 2	-0.139	0.248			
		RW	-0.324	0.008			

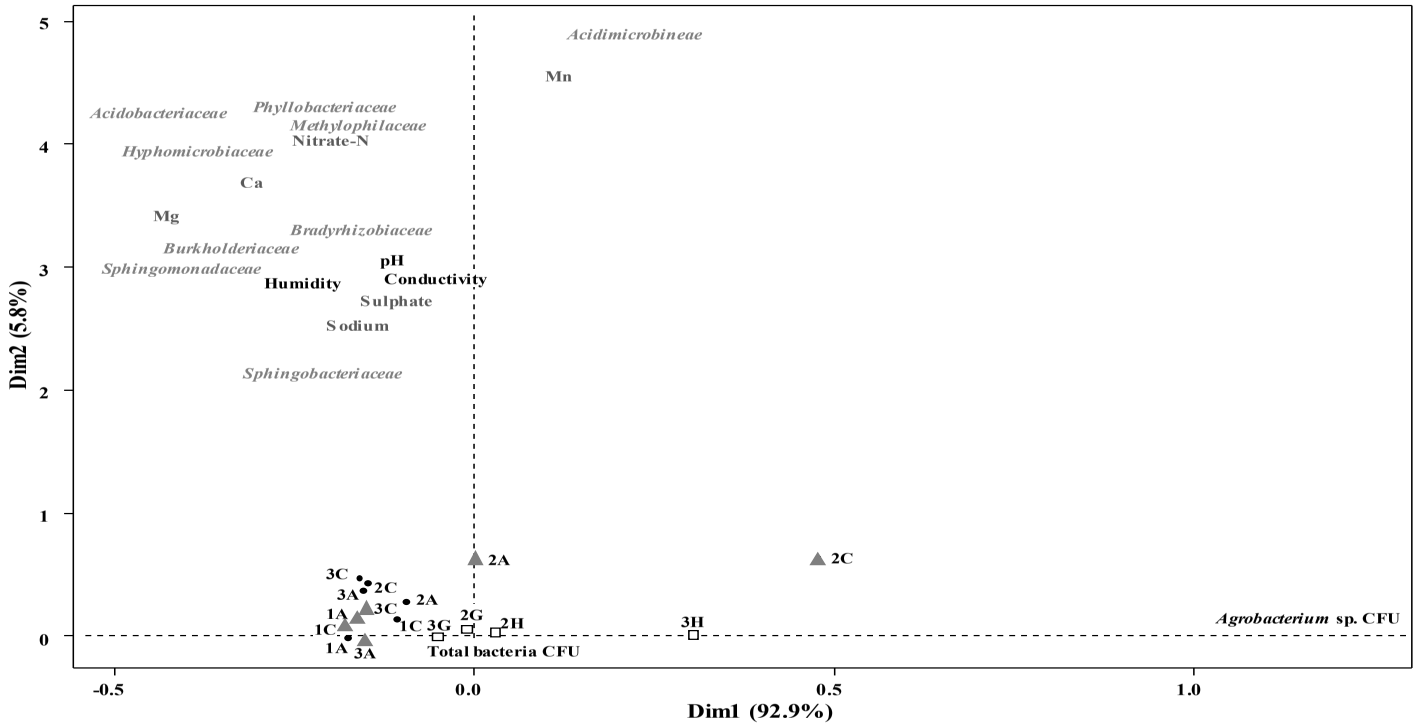
Supplementary table 2.10: CFU and PCR identification of isolates from growing media used for eggplant cultivation. GB = organic growing medium (n = 15), RW = mineral growing medium (n = 15), RWS = mineral growing medium with hairy roots (n = 10).

Growing medium	**Hairy roots**	***A. rhizogenes* bv2 (1066 bp)**	***virC* gene**	***A. rhizogenes* CFU mL^{-1}**	***P* value**	**Total bacteria CFU mL^{-1}**	***P* value**
GB	0 %	0 %	0 %	7 618 ± 200 726^{b}		6 722 636 ± 3 950 556^{a}	
RW	0 %	46.7 %	0 %	29 473 ± 200 726^{b}	0.004	10 682 474 ± 3 950 556^{a}	0.19
RWS	100 %	90 %	50 %	1 006 296 ± 234 397^{a}		17 940 649 ± 4 613 255^{a}	

*Means with the same letter are not significantly different.



Supplementary figure 2.1: Clustering based on the physical and chemical characteristics of the growing media are consistent with hierarchical clustering of bacterial profiles. UPGMA was applied to analyze the PCR-DGGE fingerprints. RW: mineral growing medium; GB: organic 55 growing medium, RWS: mineral growing medium with hairy roots. Tpt, time point.



Supplementary figure 2.2: Correspondence analysis displaying the relationships among bacterial fingerprints, bacterial relative abundances and physical and chemical characteristics of the horticultural growing media. RW: mineral growing medium; GB: organic growing medium, RWS: mineral growing medium with hairy roots. Symbols indicate the growing medium type: black circles for GB, grey triangles for RW and white squares for RWS. The number in the legend specifies the time point and the letter refers to the sample replicate. For instance, the circle labelled as "1A" refers to the replicate "A" of GB, collected at the first time point.

CHAPTER 3: GROWING MEDIA CONSTITUENTS DETERMINE THE MICROBIAL NITROGEN CONVERSIONS IN ORGANIC GROWING MEDIA FOR HORTICULTURE

This chapter has been redrafted after:

Oliver Grunert, Dirk Reheul, Marie-Christine Van Labeke, Maaike Perneel, Emma Hernandez-Sanabria, Siegfried E. Vlaeminck, Nico Boon. "Growing media constituents determine the microbial nitrogen conversions in organic growing media for horticulture." *Microbial Biotechnology* (2016).

ABSTRACT

Vegetables and fruits are an important part of a healthy food diet, however the eco-sustainability of the production of these can still be significantly improved. European farmers and consumers spend an estimated €15.5 billion per year on inorganic fertilizers and the production of N-fertilizers results in a high carbon footprint. We hypothesized, that in contrast to the individual medium constituents, a blend will create a more optimal physico-chemical microbial environment, resulting in higher ammonia and nitrite oxidation rates. Related research questions were if we can use commercial available liquid organic fertilizers and to what extent we need to adapt the N fertilization strategy and estimate the risk of ammonium toxicity. We demonstrated that growing media constituents showed differences in urea hydrolysis, ammonia and nitrite oxidation and in carbon dioxide respiration rate. Interestingly mixing of the growing media constituents resulted in a stimulation of the function of the microorganisms. The use of organic fertilizer resulted in the highest increase of bacterial *amoA* gene copy number with the highest airflow rate of 25 mL s^{-1} compared to inorganic fertilizers. The findings contribute to the understanding of the functional microbial community in growing media and its potential role towards a more responsible horticulture.

1. Introduction

Vegetables and fruits are fundamental for a healthy diet, with a recommended daily consumption of at least 400 g of both (WHO and Consultation 1990). The eco-sustainability of their production can be significantly improved. Current systems for growing vegetables are soilless culture systems (Gruda 2008). These systems guarantee stable production and high yield of quality products per unit of area, while excluding soil-borne diseases (Grillas et al. 2001; Morard 1995; Postma 2009). Growing media are used to support the development of plants and have three main functions: supply roots with nutrients, air and water, allow for maximum root growth, and physically support the plant. Air and water travel through these pore spaces and water is the medium that carries nutrients that plants need to fuel their growth, and air is needed for root growth and the health of soil microorganisms.

Growing media are formulated as a blend of different growing media constituents like peat, coir pith, wood fiber, bark, composted materials. Usually these blends are enriched with lime and inorganic or organic fertilizers in order to achieve the correct balance of physical and chemical properties. In the Netherlands and Belgium, nearly all vegetables like tomatoes, eggplants, cucumbers and peppers are grown in glasshouses using mineral horticultural growing media (Islam 2008). Unfortunately mineral growing media lack a diverse and competitive microbial community (Postma 2009). **[Chapter 2]** showed that the interactions in the resident community in GB may also play a role in the resistance to external forces, such as invasive species competing in conventional soilless culture systems. In addition we found in **[Chapter 2]** that the bacterial community inhabiting the GB medium may have higher temporal stability and resilience to this heterogeneous and fluctuating environment. However, there is limited knowledge concerning the functionality, i.e. ammonia and nitrite oxidation rate, of growing media.

Environmental and product quality concerns have prompted the search for alternative growing media (Grunert et al. 2008; Vaughn et al. 2011). Organic growing media and their constituents (Donnan 1998; Olle et al. 2012) exhibit advantages because of their diverse and competitive saprophytic microbial community, which can influence the nutrient status of the root environment (Carlile and Wilson 1990). The use of organic derived nitrogen in combination with organic growing media implies control and knowledge of the conversion of organic forms of nutrients and especially nitrogen into forms that can be taken up by the plants. All heterotrophic soil organisms consume organic substances for energy and carbon and, at the same time, immobilize and mineralize N (Robertson and Groffman 2007). Ammonium has been viewed as the immediate product of the heterotrophic mineralization. The next step of the nitrification is the conversion of ammonia (NH_3) to nitrite (NO_2^-), followed by the oxidation of nitrite into nitrate (NO_3^-). Ammonia (NH_3) and nitrite (NO_2^-) are mainly converted by chemoautotrophic microorganisms, but heterotrophic bacteria and fungi can produce NO_3^- in acid soils, thereby avoiding ammonification (Hodge et al. 2000). Mineralization of the organic-derived nitrogen in peat is completed by Ammonia Oxidizing Archaea (AOA) (Pester et al. 2012; Prosser and Nicol 2012). On the contrary, the low abundance of Ammonia Oxidizing Bacteria (AOB) because of the low NH_3 concentrations in peat, represents an advantage for the AOA. Previous reports indicated that the activity of the ammonia-oxidizing *Nitrosomonas* and the nitrite-oxidizing *Nitrobacter* in peat and peat-based growing media was positively correlated with the accumulation of ammonia and the addition of fertilizers (Bunt 2012a; Bunt 2012b). Microbial respiration rates increase when the optimal physico-chemical environment is optimal and will potentially lead to high rates of mineralization

(Robertson and Groffman 2007). The C/N ratio of the organic matter determines the availability of the carbon in the material relative to the available nitrogen. Mineralization is favored over immobilization when the organic fertilizer has a C/N ratio close to the C/N ratio of microorganisms, which is according to Based on our analysis, our best estimate of the soil microbial biomass C/N/P molar ratio is 60:7:1 (Cleveland and Liptzin 2007) or a C/N of approximately 8:1. Oxygen is required for the oxidation of ammonia and nearly all nitrifiers are obligate aerobes. However the oxygen partial pressure seems to influence the activity, rather than the diversity of AOB (Bodelier et al. 1996; Bollmann and Laanbroek 2002; Kowalchuk et al. 1998).

A drawback of many of the organic fertilizer is a fast accumulation of ammonium in peat based growing media and low nitrate contents (Raviv et al. 2005). As delivery and production of readily available nutrients derived from microbial activities of growing media constituents and its blends is difficult to predict and to control, it increases our need of investigating the potential of the microbial community associated with growing media to improve the nutrient flow in organic soilless culture systems. The microbial diversity in soil (Torsvik et al. 1990) and most likely also in soilless culture systems in combination with organic growing media is enormous and there may be substantial overlap (Chapin et al. 1997) or even a gap in function between microbial species and growing media constituents. Furthermore, it is likely that microorganisms within a functional group differ in their response to the environment. In addition soils are very well known for their priming effects and Kuzyakov (2002) defined priming effects as strong short-term changes in soil organic matter turnover caused by comparatively moderate treatments of the soil, e.g., fertilization, drying-rewetting, or mechanical disturbance or mixing. A priming effect, which can be positive or negative, occurs when a limiting factor for microbial biomass is removed, e.g., when mineral N or an easily decomposable energy source is added. Moreover, microorganisms can quickly fill out empty niches occurring when the environment is changing (Giller et al. 1998). We hypothesized, that in contrast to the individual medium constituents, a blend will create a more optimal physico-chemical microbial environment, resulting in higher ammonia and nitrite oxidation rates. Related research questions were if we can use commercial available liquid organic fertilizers and to what extent we need to adapt the N fertilization strategy and estimate the risk of ammonium toxicity. We quantified the CO_2-respiration and nitrification rate of the microbial community associated with different growing media constituents and a predefined blend in batch tests and under controlled, commercial application-like conditions in order to develop a fertigation strategy for the growth of tomatoes with organic fertilizers.

2. Material and methods

2.1 Growing medium and constituents

Organic growing medium constituents i.e. sod peat, Irish peat, coconut fiber and green waste compost (RHP certified quality) were separately tested and used as potential inoculum for nitrifying culture as described by (Saison et al. 2006). Compost was used as a control and is known as a microbial rich growing media constituent (Zeng et al. 2013) and the mineral growing medium has no microbial activity (RW, Rock wool, Grotop expert, Grodan). The blend (GB) was a mixture of sod peat (40% v/v), Irish peat (40% v/v) and coconut fiber (20% v/v). The blend (GB) was fertilized with calcium and magnesium carbonate (Dolokal Extra PG, Ankerpoort, The Netherlands) at 2.5 kg/m³ to reach pH(H_2O) of 5.5. The

fresh bulk density of the organic growing medium constituents and blend was determined according to EN 12580, i.e. without compaction. The different constituents and the final growing media were chosen, because of its excellent physicochemical properties. Previous research with plants showed that plants grown in GB, a peat-coconut fiber based growing medium resulted in similar yields like a mineral growing medium (Grunert et al. 2016a; Grunert et al. 2008) in combination with inorganic fertilizers and organic fertilizers. The mineral growing medium (RW) was used as a control in the CO_2-respiration test and the in practice simulated test (GMRS).

2.2 Indication of physico-chemical quality parameters

GMC were analyzed for physico-chemical quality parameters. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) content in the dried samples was determined with an Ankom220 Fiber Analyzer extraction unit. Based on the fractions determined according to (Van Soest et al. 1991), the biodegradation potential was calculated as the holocellulose/lignin ratio: (%hemicellulose + %cellulose)/%lignin.

Table 3.1: Indication of the physical characteristics of the predefined growing medium and constituents at the start of the experiment (n=1).

Parameters	Coconut fiber	Sod peat	Irish peat	Green waste compost	GB (20% coconut fiber; 40% Sod peat and 40% Irish peat)
Ash content (weight-% of DM)	4	1	1	75	2
Available water content ($m^3\ m^{-3}$)	0.31	0.26	0.09	0.26	0.14
DM (weight-% of fresh product)	56	53	39	57	50
Shrinkage ($m^3\ m^{-3}$)	0.23	0.20	0.21	0.23	0.20
Air content at –1kPa matric potential ($m^3\ m^{-3}$)	0.33	0.38	0.39	0.18	0.35
OM (weight-% of DM)	96	99	99	25	97
Dry bulk density (kg m^{-3})	98	76	173	481	112
Total pore space ($m^3\ m^{-3}$)	0.95	0.96	0.90	0.82	0.93
Gravimetric water content (%)	44	47	61	43	50
Volumetric water content at -1 kPa matric potential ($m^3\ m^{-3}$)	0.61	0.58	0.51	0.64	0.43
Volumetric water content at -10 kPa matric potential ($m^3\ m^{-3}$)	0.30	0.32	0.42	0.38	0.29

The ash content is the ash or mineral content on dry matter basis; the available water content is the difference in water content between -1 kPa and -10 kPa matric potential and equals the plant available water content; DM is the dry matter content; OM is the organic matter content; dry bulk density is the density of a dried sample at 103°C; the air volume is that part of the volume of a growing medium sample filled by air at a predefined suction of -1 kPa matric potential; the process of a growing medium contracting to a lesser volume when subject to water loss is called shrinkage and is the volume of the fully saturated growing medium minus the volume of the dried growing medium divided by the volume of the fully saturated growing medium; the gravimetric water content is the amount of water on fresh weight basis of the growing medium. Drying of a sample is done by placing the samples without altering the structure in an oven at 103°C to constant mass.

Moreover the determination of the physical properties, such as dry bulk density, air volume, shrinkage, the total pore space of the sample was calculated after applying -1kPa matric potential according to EN 13041. Indication of basic chemical and physical properties of the growing media and the constituents can be found in Table 3.1, Table 3.2 and Table 3.3 and Supplementary table 3.1.

Table 3.2: Indication of the chemical (cellulose, hemicellulose and lignin content) of predefined growing medium and constituents at the start of the experiment (n=1). DM= dry matter

Parameters	Coconut fiber	Sod peat	Irish peat	Green waste compost	GB (20% coconut fiber; 40% Sod peat and 40% Irish peat)
Cellulose (% DM)	40.1	47.4	31.9	8.7	34.9
Hemicellulose (% DM)	13.6	28.5	11.6	5.0	13.1
Lignin (% DM)	37.5	17.1	43.2	8.6	36.8
Biodegradation potential (holocellulose/lignin ratio)	1.4	4.4	1.0	1.6	1.3

*Table 3.3: Indication of the chemical content of the predefined growing medium and constituents at the start of the experiment (n=1). * Chemical analysis are expressed as mg L^{-1} growing medium and growing medium constituent*

Parameters	Coconut fiber	Sod peat	Irish peat	Compost	GB (20% coconut fiber; 40% Sod peat and 40% Irish peat)
pH(H_2O)	6.5	4.4	4.8	7.7	4.4
Conductivity (µS cm^{-1})	105	24.1	35.0	562	90.6
Total ammonia nitrogen (mg N L^{-1*})	1.2	1.6	4.4	3.4	6.1
Nitrate (mg N L^{-1})	0.0	0.0	0.0	81.4	6.8
Phosphorous (mg P L^{-1})	6,8	15.9	4.8	143.2	8.5
Potassium (mg K L^{-1})	117,5	15.0	27.5	1200	103.8
Calcium (mg Ca L^{-1})	77.5	315.3	297.5	1492.5	174.5
Magnesium (mg Mg L^{-1})	35.0	137.5	122.1	180	54.8
Sulphate (mg S L^{-1})	5.0	112.4	52.0	120.9	13.9
Sodium (mg Na L^{-1})	100.0	17.5	35.0	70.0	42.8
Chloride (mg Cl L^{-1})	117.3	32.3	32.3	377.8	76.8

2.3 Nitrification and respiration rates of organic growing medium constituents

Aerobic batch experiments were performed to measure the intrinsic capacity for urea hydrolysis, ammonia oxidation and nitrite oxidation. For the nitrification batch test 10 grams of Irish peat, sod peat, coconut fiber and compost resuspended in 100 mL buffer (16.7 g L^{-1} KH_2PO_4, 3.3 g L^{-1} K_2HPO_4 and 0.6 g L^{-1} $NaHCO_3$, pH = 7.1), and incubated in 250-mL Erlenmeyer flasks, in agitation (120 rpm) at 28 °C, under non-oxygen-limiting conditions (dissolved oxygen, DO concentration, ca. 6 mg O_2 L^{-1}, Vlaeminck et al. (2007). The RW was not used for the nitrification tests as it showed a CO_2-respiration comparable with the control, one chamber without growing medium. Batches were supplemented with either urea, NH_4^+, NO_2^- or NO_3^- as nitrogen sources and supplemented with $NaHCO_3$. The final concentration of the different nutrient types was 100 mg N L^{-1}. Flasks were closed with plastic caps to avoid evaporation. Contents in the flasks were allowed to sediment for 10 min before sample collection and pH, EC, DO concentration and temperature were measured. Two samples of 1 mL of the mixed liquor were collected, filtered through a 0.45-µm-pore-size filter and analyzed for the total N balance (NH_4^+-N, NO_2^--N, and NO_3^--N). Weight of the flasks was monitored to correct for evaporation if needed as a results of opening the flasks during sampling. Additional ammonia oxidation following nitrogen mineralization and ammonification was not taken into account for the growing medium constituents. Preliminary testes, showed that peat based growing medium constituents and coconut fiber without addition of other nitrogen sources released negligible amounts of N, which is in agreement with (Raviv et al. 2005). Respiration of the growing medium constituents was determined following Lundegårdh (1927). In brief, 10 g of fresh growing medium and 15 ml of a 0.4 M KOH solution were taken and placed into a sealed headspace chamber (1.5 L) stored in the dark at 28°C for 7d. As a control, one chamber without growing medium was used. Total mass of trapped CO_2 was determined by titration of the KOH solutions with 0.02N HCl. Physical characteristics were measured according to EN13041 (DIN 2012). Tests were done in triplicate and were two times repeated.

2.4 Growing media reactor systems (GMRS)

The lab-scale GMRS were set up in a dark (Figure 3.1), temperature-controlled room (28 +/- 2°C). The slabs of organic growing medium (GB) were packed in compostable plastic (EN13432) and had the following dimensions: 0.33 m × 0.2 m × 0.075 m and a volume of 4.42 L ± 0.07 L. The slabs of the mineral growing medium (RW) had the same dimensions as GB. Slabs were placed in open plastic containers of 0.39 m x 0.28 m x 0.14 m, and positioned on top of a 3 % slope, perforated (Ø 8mm) at the bottom of the lowest end. Rainwater was used as influent to supplement the different fertilizers and entered at the highest end of the slab, while the effluent of each reactor was collected in a plastic container of 10 L at the lowest end of the slab. The influent flow rate during the experimental period was 1.32 ± 0.01 L d^{-1}.

Inorganic (IF) and organic nutrient solution (OF) were supplied to the GMRS at fixed time periods (6 times a day) every three hours, for 180 s, as described in Figure 3.1. The nitrogen supply rate was the same for IF and OF (NIF = NOF). The nitrogen concentration was decreased over time in four stages (790 mg N L^{-1} – 630 mg N L^{-1} – 470 mg N L^{-1} – 315 mg N L^{-1}). Samples of the effluent were collected 0.5 h after the second feeding. The average hydraulic residence time was 0.96 h. The IF system was used as a reference and implemented as in (de Kreij 1997; Sonneveld and Voogt (2009a)). In a first test the mineral and the organic growing medium were used in combination with IF and OF. In a second test only the organic growing medium was used in combination with IF and OF. Each treatment had three replicates (n=3). Moreover in the second test air was blown at different velocities into the organic

growing medium to provide sufficient oxygen for the nitrification process (GBOF1 =0 mL s^{-1}, GBOF2 =17 mL s^{-1}, and GBOF3 = 25 mL s^{-1}).

The organic fertilizer (OF) was a commercial organic fertilizer (tomato feed from the company Plant Health Cure, Netherlands (7% N -2% P_2O_5 -3% K_2O) was used (Supplementary table 3.2). Chemical analyses revealed that the organic fertilizer mainly consisted of urea (75 %, w/w) although otherwise indicated by the supplier and other nitrogen sources like amino acids (23%, w/w). The chemical composition of the inorganic fertilizer (IF) can be found in Supplementary table 3.2. The pH of the organic fertigation solution (OF) was corrected with NaOH and increased until the pH of the organic fertigation solution (OF) was equal to IF.

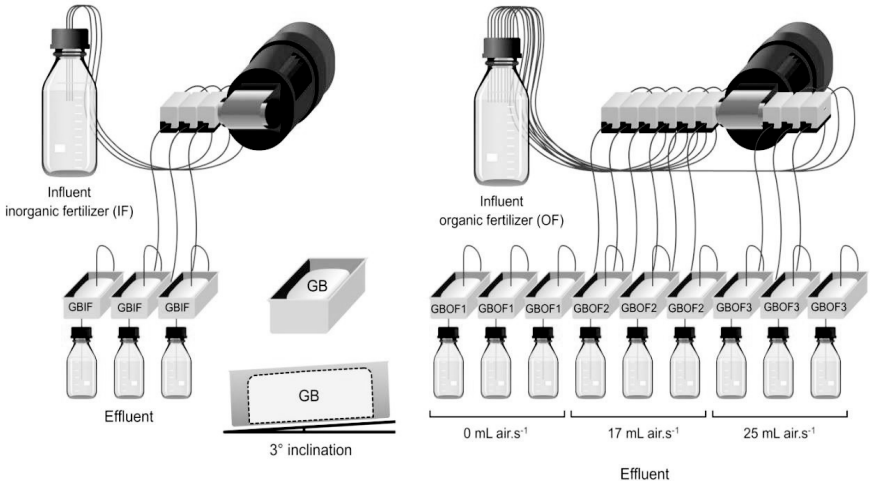


Figure 3.1: Experimental setup of the growing media reactor systems (GMRS). GB = growing bag with organic growing medium, IF = inorganic fertilizer and OF = organic fertilizer. Air was blown in the growing medium (respective airflow rates for GBOF1, GBOF2 and GBOF3 are indicated). The nitrogen supply rate was equal for all the systems: NIF = NOF. Figure was drafted by Tim Lacoere.

2.5 DNA extraction

Total DNA was extracted using physical disruption with the bead beating method from (Hernandez-Sanabria et al. 2012). Cells were lysed in a FastPrep-96 homogenisator (MP Biomedicals, Illkirch, France) and DNA was precipitated with cold ethanol and resuspended in 30 µl of TAE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). Concentration and quality of DNA were measured based on the absorbance at 260 and 280 nm in a Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.6 Abundance of ammonia oxidizers

To validate the relationships between total bacteria and AOB in the growing medium, real time PCR (qPCR) was performed on a StepOnePlus™ Real-Time PCR system (Applied Biosystems, Carlsbad, CA).

Triplicate samples of a 20-fold dilution of the DNA samples were analyzed to estimate the copy number of the *amoA* of the above bacterial group, following the procedures described by Øvreås et al. (1997) and Rotthauwe et al. (1997). For the total bacteria (V3 region) the 338F and the 518R primers were used according to Øvreås et al. (1997) and for the AOB the *amoA*-1F and the *amoA*-2R was used according to Rotthauwe et al. (1997). The reaction mixture of the 25µL was prepared by mans of the Thermo Scientific Fermentas PCR master mix (2X) and consisted of 12.5ul of 2x master mix, 1ul of each Primer

5ul of template DNA and 5.5ul water. The PCR condition was 95°C for 10 min, followed by 40 cycles of 30 s at 95°C, 60 s at 56°C and 30 s at 72°C. For every cycle, the fluorescence signal capture was at 78 °C for 15 s. The qPCR data were represented as copies per ng^{-1} DNA. The copy numbers of total bacteria were normalized according to the DNA concentrations to express in gene copies ng^{-1} DNA. The proportion of the AOB was estimated after dividing the total copy number of 16S rRNA gene by the copy numbers of the targeted gene.

2.7 Physicochemical analyses of the influent and effluent of the GMRS and of the growing media and constituents

The concentrations of total ammoniacal nitrogen (TAN = NH_4^+-N + NH_3-N) and total Kjeldahl-N (TAN + organic nitrogen) were determined as in (Anonymous 1992; Bremner and Keeney (1965)). Influent and effluent were subjected to determination of nitrite and nitrate concentrations using an ion chromatograph (Metrohm, 930 compact IC flex, Herisau, Switzerland) and dissolved oxygen (DO) was measured with a portable meter (Hach Lange). Chemical oxygen demand (COD) was determined using the Photometer Nanocolor 500 D kit, following the manufacturer's instructions (Marcherey-Nagel). Chemical characteristics of the growing media were measured according to Gabriels et al. (1998b). Potassium, phosphorus, calcium, magnesium, iron and manganese were extracted (1:5 vol/vol) in ammonium acetate and measured with ICP. The electrical conductivity (EC), pH(H2O), ammonium (NH_4^+), nitrate (NO_3^-), sulphate (SO_4^{2-}) and sodium (Na) were measured in a 1:5 v/v water extract according to EN 13038, EN 13037 and EN 13652, respectively. Nitrate was measured with an IC ion chromatograph. Ammonium was measured by steam distillation.

Carbohydrate analyzes of the samples was performed as described by Van Soest et al. (1991). Neutral detergent fiber (NDF) measures the hemicellulose, cellulose and lignin contained in the plant cells, while the residue remaining after boiling NDF in acid detergent solution is called acid detergent fiber (ADF) and contains the cellulose, lignin and ash present in the samples. Results were expressed as percentage of the dry matter (DM). C/N ratio, organic matter (OM) content (EN13039, (CEN), total nitrogen (13654-2 2001) and dry matter content (British Standard 2000) of the different constituents were determined.

2.8 Statistical analysis

Nitrification and CO_2-respiration rates and log transformed *amoA* gene copy numbers were compared by one-way analysis of variance followed by All Pairwise Multiple Comparison Tukey to check for quantitative variance between different treatments with a confidence interval of 95%. All analyzes were conducted using SPSS version 22.0 (IBM Co., Armonk, NY, USA), and $P \leq 0.05$ was considered to be statistically significant. When the data were not normally distributed ($P<0.05$) and or there was no

equal variance (P<0.05) then we used the Kruskal-Wallis One Way Analysis of Variance on Ranks followed by an All Pairwise Multiple Comparison Dunn's test.

3. Results

3.1 CO_2-respiration rate of different growing media constituents and growing medium

Mean rates of growing medium and growing medium constituents CO_2-respiration were all significantly different (*P=0.009*) between the tested growing media and constituents (Table 3.4).

*Table 3.4: CO_2-respiration rate of different fresh growing media constituents and a blend (GB) of the different growing media constituents (n=6). Different letters next to the numbers indicate a significant difference (P<0.05). ND= not determined. Stdev= standard deviation. *The C/N ratio of GB was calculated based on the C/N ratio of the individual growing medium constituents.*

Growing medium (RW and GB) and constituents	CO_2-respiration rate (mg CO_2-C kg^{-1} d^{-1}) (mean±stdev)	C/N ratio
Mineral growing medium (RW)	-2.3±9.6 [a]	3
Compost (green waste)	138.8±20.1 [e]	16
Coconut fiber	82.6±4.0 [d]	103
Sod peat	25.2±1.5 [b]	61
Irish peat	16.1±3.1 [a]	49
Blend (GB) (20% coconut fiber; 40% Sod peat and 40% Irish peat)	45.9±5.6 [c]	65*

The Irish peat (10-30 mm) showed the lowest rate of CO_2-respiration with 16.1±3.1 mg CO_2-C kg^{-1} d^{-1}, followed by sod peat (10-30 mm) at 25.2±1.5 mg CO_2-C kg^{-1} d^{-1} and coconut fiber at 82.6±4.0 mg CO_2-C kg^{-1} d^{-1}. The blend of the three growing media constituents (40 vol.% sod peat 10-30 mm, 40% Irish peat 10-30 mm and 20% coconut fiber, v/v) showed higher CO_2-respiration rate of 45.9±5.6 mg CO_2-C kg^{-1} d^{-1} than calculated based on the volume ratios used in the mixture: 33 mg CO_2-C kg^{-1} d^{-1}.

Mixing the growing media constituents resulted in an increased CO_2-respiration rate (Table 3.4) in comparison to the theoretically calculated CO_2-respiration rate. The CO_2-respiration rate of the mineral growing media was equal to the control (-2.3±9.6 mg CO_2-C kg^{-1} d^{-1}) and the CO_2-respiration rate of compost was 138.8±20.1 mg CO_2-C kg^{-1} d^{-1}. The C/N ratio was the highest for the coconut fiber and lowest for the mineral growing medium (Table 3.4).

3.2 Nitrogen transformation rates of the organic growing medium constituents

Batch activity tests were performed during 37 days. The growing medium constituent influences the ureolysis, the ammonia oxidation and the nitrite oxidation rates and the nitrogen form (urea, ammonium, nitrite and nitrate) used (Figure 3.2). The ureolysis (P<0.05), the ammonia oxidation (P< 0.05) and the nitrite oxidation rate (P<0.05) were significantly different between growing media constituents.

Urea hydrolysis ranged between 162.4±17.1 mg N kg^{-1} d^{-1} and 85.6±19.7 mg N kg^{-1} d^{-1}. Sod peat showed the highest and Irish peat the lowest rate of ureolysis. Ammonia oxidation rate ranged between 86.9±9.9 mg N kg^{-1} d^{-1} and 1.0±0.9 mg N kg^{-1} d^{-1} and was the highest in compost and the lowest in coconut fiber. Nitrite oxidation rate was the highest in compost (83.8 ± 1.3 mg N kg^{-1} d^{-1}) and lowest in the Sod peat (8.4 ± 6.2 mg N kg^{-1} d^{-1}). When growing media constituents were mixed, ammonia oxidation rate increased from 41 mg N kg^{-1} d^{-1} to 83 mg N kg^{-1} d^{-1} and nitrite oxidation rate increased from 15 mg N kg^{-1} d^{-1} to 63 mg N kg^{-1} d^{-1}.

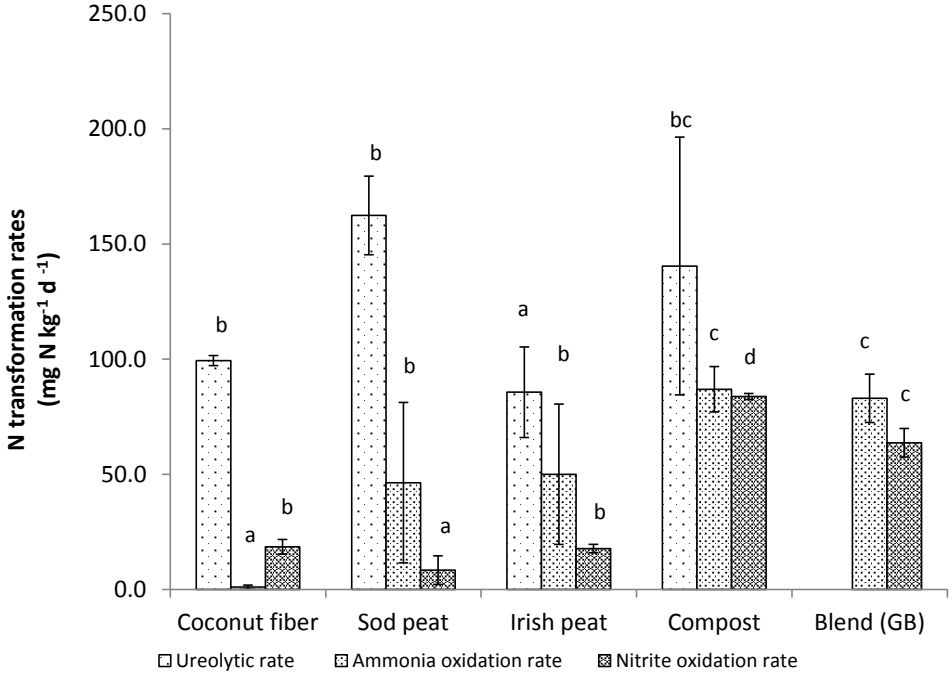


Figure 3.2: Nitrogen transformation rates by growing media constituent and blends and N treatment. Bars represent standard deviation of triplicate samples. Different letters above the bars indicate a significant difference (P ≤ 0.05). Differences should be compared within ureolytic, ammonia oxidation and nitrite oxidation rate and between different GMC and mixture (GB).

3.3 Development of a fertigation strategy with organic fertilizers suitable for the growth of tomatoes

Based on the results obtained in the different batch tests, we developed a fertigation strategy in an in practice simulated environment. First we tested the mineral (RW) and organic growing media (GB) with an average nitrogen concentration of 650 -700 mg N L^{-1} for the organic fertilizer (OF) and inorganic fertilizer (IF) (Supplementary figure 3.1). The nitrate concentration in the treatment with IF showed the same tendency for RW and GB and influent and effluent concentration were almost the same for GB and RW. On the contrary, nitrate concentrations in the treatment with OF were 95% lower in

comparison with IF, however increased nitrate concentrations were found in GB after 14-16 days (32 mg NO_3^--N L^{-1}) and 0 mg NO_3^--N L^{-1}) was found in RWOF approximately after 18 days. Moreover the concentration of the dissolved oxygen (DO) in the IF treatment remained stable (6-8 mg O_2 L^{-1}), whereas DO values in the OF treatment varied between 0-2 mg O_2 L^{-1} (Supplementary figure 3.2). The treatment with OF resulted in increased amounts of TAN (652 mg N L^{-1} for RWOF and 360 mg N L^{-1} for GBIF). The pH(H_2O) of the RWOF was about 8.7, which is far to alkaline for optimal plant growth, and 7.8 for GBOF. Free ammonia levels were 10 times higher for RWOF in comparison with GBOF (Table 3.5).

Table 3.5: Average chemical composition of the effluent of the inorganic and organic nutrient solution at the end of the experiment, i.e. 20 days of operation. n=3. Numbers are mean ± standard deviation. FA= free ammonia, FNA = free nitrous acid, Org-N= organic nitrogen, TAN= Total ammonia nitrogen, RWIF = mineral growing medium in combination with inorganic fertilizer, GBIF = organic growing medium in combination with inorganic fertilizer, RWOF = mineral growing medium in combination with organic fertilizer, GBOF = organic growing medium in combination with organic fertilizer

Treatment	Org N (mg N L^{-1})	TAN (mg N L^{-1})	N-NO_2^- (mg L^{-1})	N-NO_3^- (mg N L^{-1})	Total N (mg N L^{-1})	pH(H_2O)	FA (mg N L^{-1})	FNA (mg N L^{-1})
RWIF	0.0±0	66.9±1.9	0.0±0	631.0±31.2	697.9±32.3	7.1±0.1	0.35	0.0000
GBIF	0.0±0	64.3±10.8	0.9±1.7	687.5±64.7	752.7±75.1	6.2±0.3	0.05	0.0012
RWOF	22.0±14.6	652.9±52.1	0.0±0	0.0±0	674.9±53.6	8.7±0.5	123.9	0.0000
GBOF	5.3±10.3	360.5±142.8	21.9±7.5	51.2±16.5	438.9±155.6	7.8±0.3	11.4	0.0008

In a second experiment we used the blend (GB) in combination with OF to further optimize the fertigation strategy (Supplementary figure 3.3) and it was decided not to test RWOF due to very high pH values (pH(H_2O =8.7) and too high ammonia levels (653 mg N L^{-1}). In the second experiment the nitrogen concentration was gradually decreased from 790 mg N L^{-1} (days 0-29) to 635 mg N L^{-1} (days 30-39), 477 mg N L^{-1} (days 40-48) and 320 mg N L^{-1} (days 49-55).

We observed that during the fourth period with the lowest organic nitrogen supply rate (operational day 49-55) on average 22.5% NH_4^+ was formed per mg organic nitrogen. This strategy resulted in increased nitrate concentrations with low free ammonia level (0.5 mg N L^{-1}) and with the highest dissolved oxygen content (DO) (5.05 mg O_2 L^{-1}) in the effluent. Also the pH(H_2O) decreased from 8.2 to 7.6 and the electrical conductivity decreased from 3.7 mS cm^{-1} to 1.3 mS cm^{-1}. Blowing extra air into the growing media (GBIF - 0 mL s^{-1}, GBOF1 – 0 mL s^{-1}, GBOF2 - 17 mL s^{-1} and GBOF3 - 25 mL s^{-1}) did not increase the ammonia and nitrite oxidation rates.

3.4 Comparison of the abundance of the amoA gene copy number between growing media, fertilizer and aeration treatments

Figure 3.3 shows the changes in abundance of bacterial *amoA* genes of the organic growing medium treated with organic or inorganic fertilizer. We determined that the AOB *amoA* gene copy number was below detection limit in both fresh growing media at the start of the test. The copy number of the total 16S rRNA genes was below the detection limit (<10^3 copies g^{-1} of growing medium) for the mineral media (RW) and 1.5x10^5 ± 2.3×10^4 copies g^{-1} of the blend (GB). Growing media with different fertilizer

regimes were analyzed after 55 days by qPCR. The percentage of the bacterial copy number of *amoA* gene over de total 16S rRNA gene copy number was 0.12% in the GBIF treatment, 1.17% in the GBOF1, 1.02 % in the GBOF2 and 0.90% in the GBOF3 treatment. Blowing air into the growing medium and the use of organic fertilizer had a significant positive effect on the bacterial *amoA* gene copy number ($P< 0.001$) and the total bacteria ($P< 0.001$), however the ratio *amoA* gene copy number over total 16S rRNA gene copy number remained stable with increasing air flow.

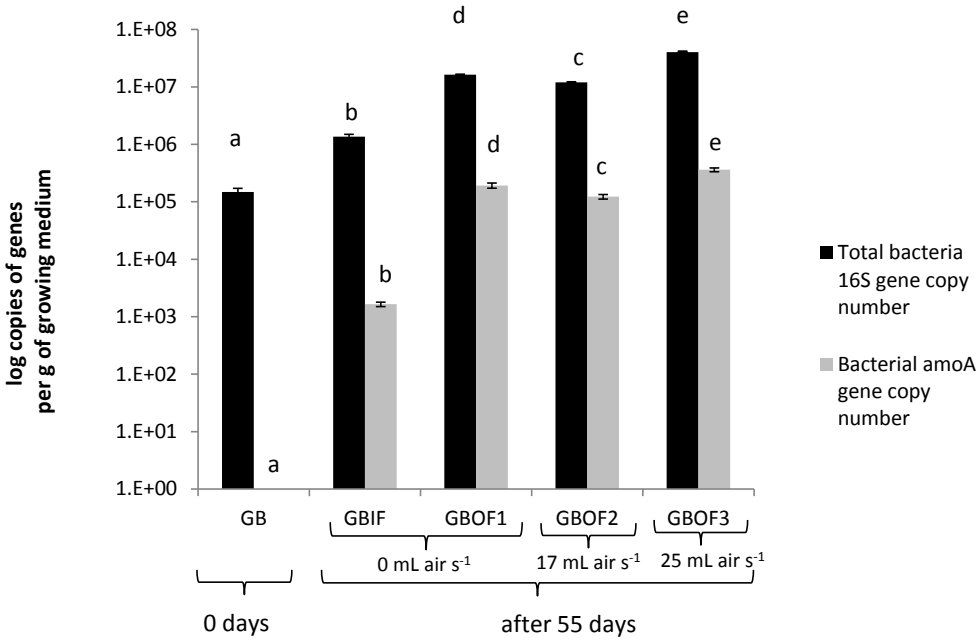


Figure 3.3: Changes in abundance of bacterial amoA genes and total bacteria organic growing media (GB) in combination with inorganic fertilizer (IF) and organic fertilizer (OF) determined at the end of the experiment, i.e. 55 days of operation. Bars represent standard deviation of triplicate samples (n=3). Different letters above the bars indicate a significant difference ($P < 0.05$*). GBOF1: organic growing medium with organic fertilizer (0 mL air s^{-1}); GBOF2: organic growing medium with organic fertilizer (17 mL air s^{-1}); GBOF3: organic growing medium with organic fertilizer (25 mL air s^{-1}).*

4. Discussion

We hypothesized, that in contrast to the individual medium constituents, a blend will create a more optimal physico-chemical microbial environment, resulting in higher ammonia and nitrite oxidation rates. Our study provides evidence that growing medium constituents and an adapted fertigation strategy determine microbial nitrogen conversions in organic growing media for horticulture. Moreover, we can accept the hypothesis that the ammonia and nitrite oxidation rate of the community associated with the individual growing medium constituents is positively correlated to the ammonia and nitrite oxidation rate of the blend of these individual constituents and can be used as a growing

medium in combination with organic fertilizers. In addition, we can use commercial available liquid organic fertilizers, however we need to adapt the N fertilization strategy to avoid the risk of ammonium toxicity. Hence, we can blend microbial communities with a specific function, such as ammonia and nitrite oxidation, associated with growing media constituents in a growing medium to achieve nitrification in the final blend. This strategy can be used as a next step towards a more sustainable horticulture in combination with soilless culture systems, where the delivery and production of organic-derived nutrients can be predicted and controlled in a more reliable way.

4.1 Effect of blending in relation to respiration rates

The respiration of soil and potentially also growing media are influenced by environmental factors, such as water content, oxygen supply, total pore space, air content, nutrient content, pH (Ryan and Law, 2005). We found, that blending of growing medium constituents has a positive effect on the respiration of the final blend. This effect was until now not described in soilless culture systems and growing media, however this "priming effect" is very well known in soil (Kuzyakov 2002). A priming effect appears, when a limiting factor for the microbial community is eliminated. In general, the priming effect arises either immediately or very shortly after the addition of a specific substance to the soil (Dalenberg and Jager 1981; 1989; Kudeyarov 1988). As described by Kuzyakov (2002), these positive priming effect with respect to carbon (C) is coming from a surplus decomposition of organic C after addition of easily-decomposable organic substances to the soil (Dalenberg and Jager 1989) or growing media. This results in the acceleration of the microbial community turn-over and a CO_2 flush from the microbial community. As shown by Fontaine et al. (2003) it is very likely that nutrient-poor soils are more often subjected to the priming effect than nutrient-rich soils. Indeed, we used GMC (Irish peat, sod peat and coconut fiber) with a low nutrient composition (Table 3.3) and a different cellulose, hemicellulose and lignin content and consequently biodegradation potential. The physico-chemical characteristics and the biodegradation potential of the blend were different compared to the individual constituents (Table 3.2).

Hamer et al. (2009) hypothesized that N deficiency favors the priming effect when mineral N is supplied. Indeed, N limiting conditions are indicated by the C/N ratio of the individual GMC. The microbial community of the blend encounters a different C/N ratio compared to the individual constituents indicating an environment with a different carbon and nitrogen availability. Priming effects are caused by comparatively moderate treatments, such as fertilization and mechanical disturbance (Kuzyakov 2002) or blending of growing media constituents. Mixing different GMC influences the chemical and the physical composition (Table 3.1, Table 3.2 and Table 3.3, Supplementary Table 3.1) of the blend (GB) and can be considered as a "fertilizer effect". This fertilizer effect might explain the increase in respiration rate of the blend compared to the different growing media constituents.

The molar C/N ratio of the microbial biomass at a global scale converges towards 6–8 (Cleveland and Liptzin 2007). Microorganisms decomposing organic matter with a higher C/N ratio than 6-8 are confronted with a surplus of C in relation to N and microorganisms confronted with a lower C/N ratio are facing a lack of C in relation to N (Spohn and Chodak 2015). During composting microorganisms transform organic matter into CO_2, biomass, heat and humus-like end-product. The high respiration rate of compost can be explained by the fact that C/N ratios lower than 30:1 allow rapid microbial growth and fast decomposition, while the tested growing medium constituents all have a higher C/N

ratio indicating slower microbial growth and a decreased decomposition of the constituents. As composting proceeds, the C/N ratio gradually decreases from 30:1 to 10–15:1 for the finished mature product. This occurs because each time that organic compounds are consumed by microorganisms, two-thirds of the carbon is lost to the atmosphere as CO_2 gas, while most of the nitrogen is recycled into new microorganisms. The Irish peat and sod peat have a C:N ratio in the range of 12–80, which is similar to soil litter (Berg and McClaugherty 2003), coconut fiber has an even higher C:N ratio. When growing on N-poor growing media, microorganisms have not enough N to build up as much biomass as the C concentration would allow resulting in decreased respiration rates in comparison to green compost. The biodegradation potential of the different constituents is quite similar (Table 3.2), except for the sod peat, indicating that the nitrogen form and nitrogen concentration present in the individual growing medium constituents plays a crucial role (Table 3.3) and might explain the differences in the carbon respiration rate.

4.2 Effect of blending in relation to potential nitrogen conversion rates

The peat based GMC had a significant higher ammonia oxidation rate compared to the coconut fiber, however significantly lower than green waste compost. Coconut fiber and Irish peat had a significant higher nitrite oxidation rate than sod peat, however it was significantly lower than green waste compost. The reported values are higher than the values reported by Stopnišek et al. (2010). They found net nitrification rates reaching 13.8 mg NO_3^--N kg^{-1} soil d^{-1}, which is in the upper range of *in situ* gross nitrification rates analyzed in a metastudy of approximately 100 different soils (Booth et al. 2005). We, on the contrary, found potential ammonia oxidation rates of 86 mg NH_4^+-N kg^{-1} growing medium d^{-1}. Nitrite rarely accumulates in soil, and studies showed that the nitrite concentration is negligible in relation to nitrate concentration (Stopnišek et al. 2010). These differences can be explained by the fact, that our tests were executed at higher pH(H_2O)= 7.1 instead of pH(H_2O)=4, higher incubation temperatures were used 28°C instead of 22°C and potential mineralization of organic nitrogen present in the GMC was not taken into account. On the other hand, ammonia oxidation rates could be underestimated due to immobilization of the ammonium by the organic material. Moreover we estimated the *ex situ* potential rates and not the *in situ* gross nitrification rates (Stopnišek et al. 2010). Next to a positive effect of the blending approach on the respiration rates, we also found a positive effect of the blending (GB) on the ammonia and nitrite oxidation rates in comparison to the calculated rates. Indeed, priming effect were not only found for C, but also for N, and other elements (Kuzyakov 2002). This priming effect with respect to nitrogen is defined as every effect on N already in the soil or growing medium by adding N to the soil or growing medium (Jenkinson 1966). Indeed, according to Kuzyakov (2002) the addition of easily accessible organic substances and the addition of mineral N nutrients as a result of the blending, can activate the microbial community, explaining this positive priming effect. It is very well known, that ammonia causes larger priming effects than nitrate (Kuzyakov 2002; Rennie and Rennie 1973). The physical properties of the growing medium constituents and the growing medium may also influence priming effect due to sorption of nutrients or even immobilization of nutrients.

4.3 Effect of organic growing media in relation to N dynamics in an in practice simulated experiment

GB showed high potential ammonia and nitrite oxidation rates. The organic nitrogen concentration blended in growing media needs to be in accordance with the ammonia and nitrite oxidation rate of

the microbial community associated with the growing medium constituents (Munch and Velthof 2006). The mineral growing medium showed the lowest respiration rates indicating very low or even no microbial activity. Consequently, RW is a less optimal growing medium in combination with organic fertilizers. GB, on the contrary, had a potential ammonia oxidation rate of 83 mg NH_4^+-N kg^{-1} growing medium d^{-1}. The applied organic nitrogen supply rate of 860 mg N kg^{-1} growing medium d^{-1} was 10x higher than the potential ammonia oxidation rate, however nitrogen was supplemented gradually in little shock loads. The results from the GMRS indicate a rapid accumulation of TAN in the effluent in combination with the highest nitrogen load, suggesting an incomplete nitrification with low or no nitrate production. These results suggest a possible inhibition of the nitritation by free ammonia due to increased pH values and high ammonium concentrations (Supplementary figure 3.3). Consequently, the supplied organic derived nitrogen is not in accordance with the ammonia oxidation rate, which is the rate limiting step, of the used blend.

When the nitrogen concentration was gradually decreased until 315 mg N L^{-1}, nitrate concentrations increased gradually (Table 3.5). Nitrate production was highest when the organic loading rate was below 315 mg N L^{-1} with acceptable pH(H_2O)=7, electrical conductivity (1000 µS cm^{-1}) and DO (4–5 mg O_2 L^{-1}, indicating that the supplied organic derived nitrogen is more in accordance with the ammonia oxidation rate. Based on the results obtained, it can be calculated that the microbial community is exposed to a nitrogen supply rate of 72 mg N kg^{-1} growing medium per shock load. From the results obtained, it became obvious that the supplied organic derived nitrogen must be as close as possible in accordance with the ammonia oxidation rate indicated by the production of nitrate.

4.4 Effect of organic fertilizer in relation to bacterial *amoA* abundance.

The practice simulated experiment with the blend showed that the fertigation with IF and OF increased total bacteria and *amoA* copy number per g of growing medium. Also Shen et al. (2008) found the highest bacterial *amoA* gene copy numbers in those treatments receiving N fertilizer. Moreover, we saw that bacterial *amoA* gene copy number was increased depending on the N fertilizer type used. Use of OF increased the *amoA* copy number 100 times higher ($P< 0.001$) in comparison to IF, when 25 mL air s^{-1} was blown in the growing medium. Chemolithotrophic ammonia-oxidizing bacteria are adapted to oxygen limitation with respect to their affinity for oxygen, ability to survive periods of anoxia, and immediate response to the appearance of oxygen (Kowalchuk et al. 1998). The non-responsiveness of the ammonia oxidizing bacteria to an airflow of 17 mL air s^{-1} might be explained by the too low flow rate to fully oxygenate the entire growing medium. The treatment with the highest bacterial *amoA* gene copy number showed also the highest nitrate formation rates. The abundance of AOB are in agreement with the results of Wang et al. (2014) in a long-term manure fertilized soil ($8.8\ 10^5$ to $3.2\ 10^8$ g $^{-1}$ dry soil). Wu et al. (2011) and Jia and Conrad (2009) found a stimulating effect of manure fertilization to the population of AOB rather than AOA. Moreover, as shown by Verhamme et al. (2011) ammonia oxidizing archaea (AOA) grow at low, intermediate and high ammonia input. AOB, on the contrary, only grow significantly at high ammonia concentrations (200 µg NH_4^+ -N per gram of soil). The results, obtained under controlled conditions, are consistent with field observations. The data also suggest that growth of AOB is favored at high ammonium concentration, which is more typical of agricultural soils receiving high inorganic nitrogen input (Verhamme et al. 2011) or in soilless culture systems in combination with organic fertilizer and organic growing media.

4.5 Blend in relation to a potential nitrifying community

Chemoautotrophic microorganisms mainly convert ammonium (NH_4^+) and nitrite (NO_2^-). Genuinely archaeal and bacterial ammonia oxidizers (AOA and AOB) drive nitrification and appear to be ubiquitous, suggesting activity across the pH(H_2O) range investigated. The different growing media constituents have different chemical and physical properties (Table 3.1, Table 3.2, Table 3.3 and Supplementary Table 3.1). Peat has a pH(H_2O) of about 4.5 and AOA seem to be predominant in acidic forest soils, tea soils or peat (Stopnišek et al. 2010) and, AOB are often found to dominate in soil with greater inorganic N availability (Thion et al. 2016). Ammonia is a direct substance for ammonia oxidizers (Suzuki et al. 1974), so acidic growing media constituents might be perceived as ammonia-limited oligotrophic environments, due to exponential ionization of ammonia to ammonium with decreasing pH(H_2O) (De Boer and Kowalchuk 2001; Zhang et al. 2012b). The initial ammonium concentrations are relatively low in our two acidic growing media constituents ranging from 2 to 7 mg N L^{-1} growing media, and consequently the ammonia concentrations based on the ionization equilibrium in soil water will be even lower. Coconut fiber on the contrary can be considered as neutrophilic (pH(H_2O) = 6.5) and has a high lignin content. White-rot fungi are known to break down lignin with the aid of extracellular peroxidase and laccase enzymes. There are also reports of bacteria that can degrade lignin (Bugg et al. 2011). Indeed a wide variety of heterotrophic bacteria and fungi can oxidize ammonium and heterotrophic nitrification appears in some soils (Robertson and Groffman 2007) and probably also growing media. Ureolytic growth of AOB also occurs at low pH(H_2O) (Burton and Prosser 2001; de Boer and Laanbroek 1989), which might indicate ureolytic activity in sod and Irish peats. The enrichment of the first acidophilic, autotrophic, ammonia oxidizer, *Nitrosotalea devanaterra*, provides an explanation for nitrification in acidic soils (Lehtovirta-Morley et al. 2011). Although NOB prefer a neutral pH(H_2O)-value as we have in coconut fiber, Hankinson and Schmidt (1988) succeeded in isolating a strain of *Nitrobacter* growing at pH(H_2O) 5.5. Indeed, we found that the potential rates for ammonia oxidation and nitrite oxidation were higher for the peat-based constituents, however, coconut fiber showed higher nitrite oxidation rates (Figure 3.2). The results indicate that we have different nitrifying communities in the different growing media constituents. Consequently, blending these constituents and its different nitrifying communities with each other indicates that we potentially have a mix of a heterotrophic and autotrophic nitrifying community in the mixture (GB).

4.6 Practical aspects of this study allowing for fine-tuning N fertilization in soilless culture systems

These results indicate that the maximum organic nitrogen concentration in soilless culture systems in combination with organic growing media should not be higher than 315 mg N L^{-1}. The ratio between ammonium and nitrate was 2:1 and the ammonium concentration was about 70 mg N L^{-1}. Moreover, the supply of oxygen did not have a positive effect on the nitrate concentration. The typical inhibiting effect of ammonium on AOB and NOB might be counteracted by accomplishing a gradual increase of the applied shock loads. These results indicate that the use of organic fertilizers in combination with soilless culture systems and organic growing media, require loads close to the ammonia oxidation rate of the blend (83 mg N kg^{-1} d^{-1}) with a maximum concentration of 315 mg N L^{-1} in order to allow a community shift towards a more adapted nitrifying community.

5. Conclusion

We identified in Grunert et al. (2016a) that *Nitrospira* and the *Nitrosomonadaceae* were closely related to GB. In fact, the *Actinomycinae*, which was highly correlated with the GB in the first time point, had significant correlation with the Nitrospira family. Moreover, *Alcaligenaceae* has significant correlation with the GB in the third time point. Hence, we identified nitrifying communities that are closely related to GB. Due to the scope of the work, the above identified nitrification and respiration rates between different growing media and constituents represent the basis for future research. Additional growing media constituents and organic fertilizers need to be investigated for their potential application in horticultural settings and more detailed information is needed on the respiration, and ammonium and nitrite oxidation rate of various blends and constituents. These results contribute to the understanding of the functional microbial community and its role towards microbial management of organically derived nitrogen in peat-based growing medium.

6. Acknowledgements

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Supplementary information

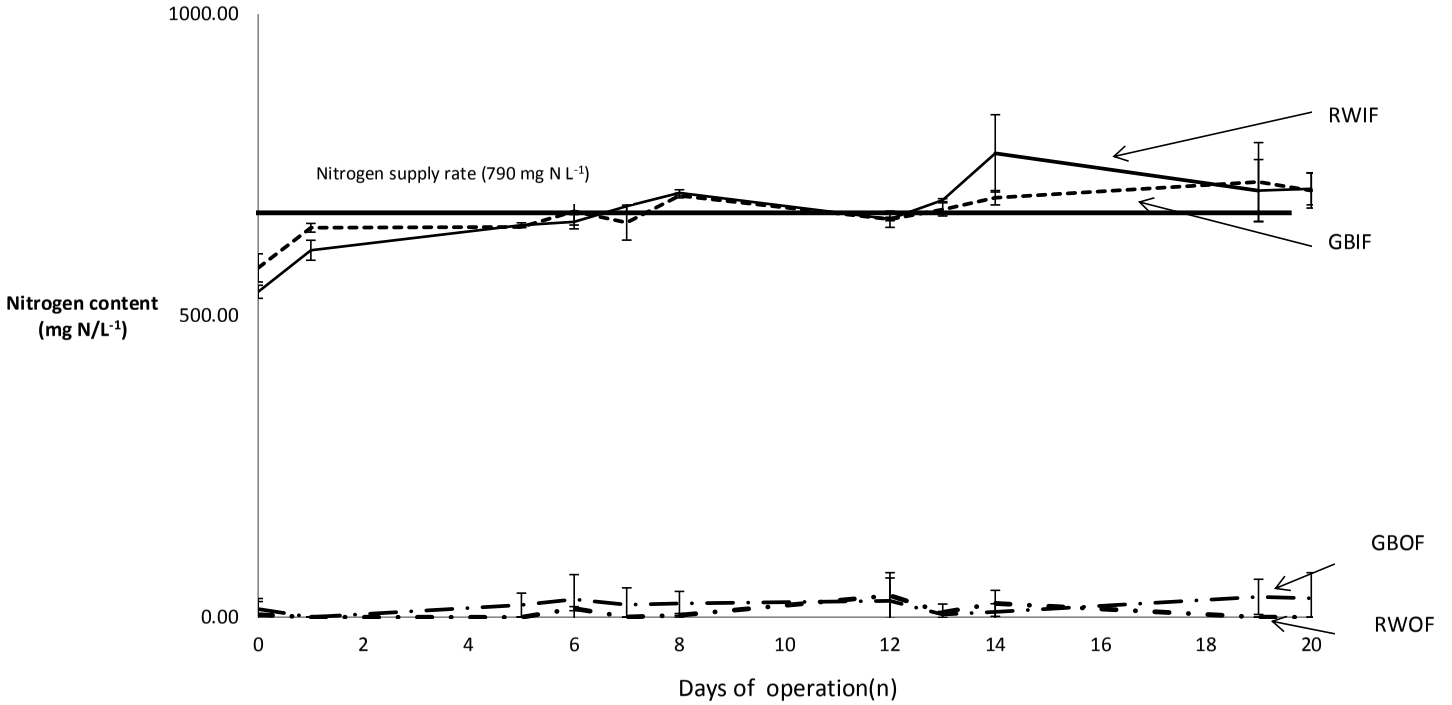
Supplementary table 3.1: Chemical analyses of unused mineral and organic growing medium without lime addition (n=10).

*NS= not significantly different. (P <0.05). * Chemical analysis are expressed as mg L^{-1} growing medium*

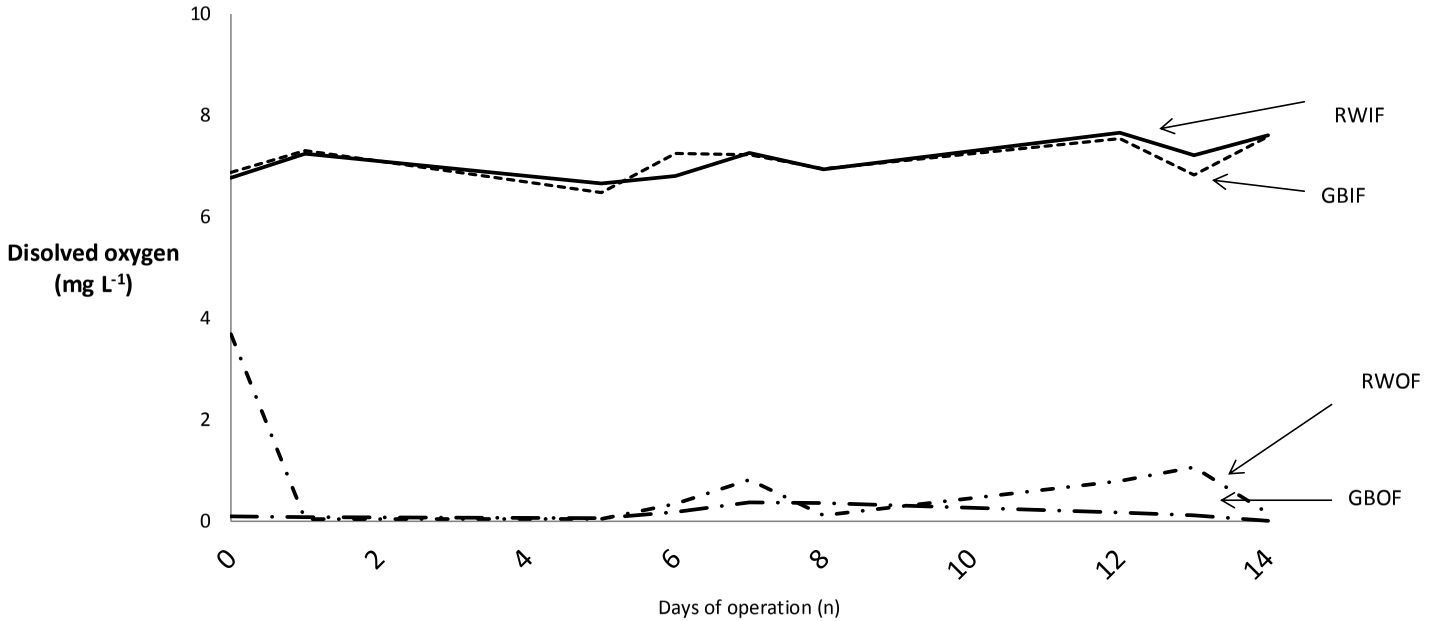
Variable	Growing medium		P value
	Mineral growing medium (RW)	Organic growing medium (GB)	
pH(H_2O)	6.30 ± 0.18	4.40 ± 0.10	< 0.001
Conductivity (µS cm^{-1})	20.09 ± 1.66	90.63 ± 20.99	< 0.001
Organic nitrogen (mg N L^{-1*})	2.13 ± 2.71	0.32 ± 0.55	NS
Total ammonia nitrogen (mg N L^{-1})	0.51 ± 0.13	6.05 ± 0.51	< 0.001
Nitrite (mg N L^{-1})	0.00 ± 0.00	0.00 ± 0.00	< 0.001
Nitrate (mg N L^{-1})	0.00 ± 0.00	6.78 ± 5.14	0.001
Phosphorous (mg P L^{-1})	1.43 ± 0.25	8.51 ± 1.31	< 0.001
Potassium (mg K L^{-1})	6.25 ± 2.12	103.75 ± 31.43	< 0.001
Calcium (mg Ca L^{-1})	22.25 ± 14.74	174.5 ± 28.72	< 0.001
Magnesium (mg Mg L^{-1})	6.25 ± 1.77	54.75 ± 5.46	< 0.001
Sulphate (mg S L^{-1})	7.28 ± 2.01	13.85 ± 3.01	< 0.001
Sodium (mg Na L^{-1})	9.00 ± 1.29	42.75 ± 9.16	< 0.001
Chloride (mg Cl L^{-1})	3.42 ± 0.88	76.80 ± 24.43	< 0.001
Iron (mg Fe L^{-1})	4.13 ± 0.41	0.10 ± 0.03	< 0.001
Manganese (mg Mn L^{-1})	0.13 ± 0.03	0.60 ± 0.11	< 0.001

Supplementary table 3.2: Chemical composition of the organic (OF) and inorganic nutrient solution (IF) used in the growing media reactor system (GMRS) tests.

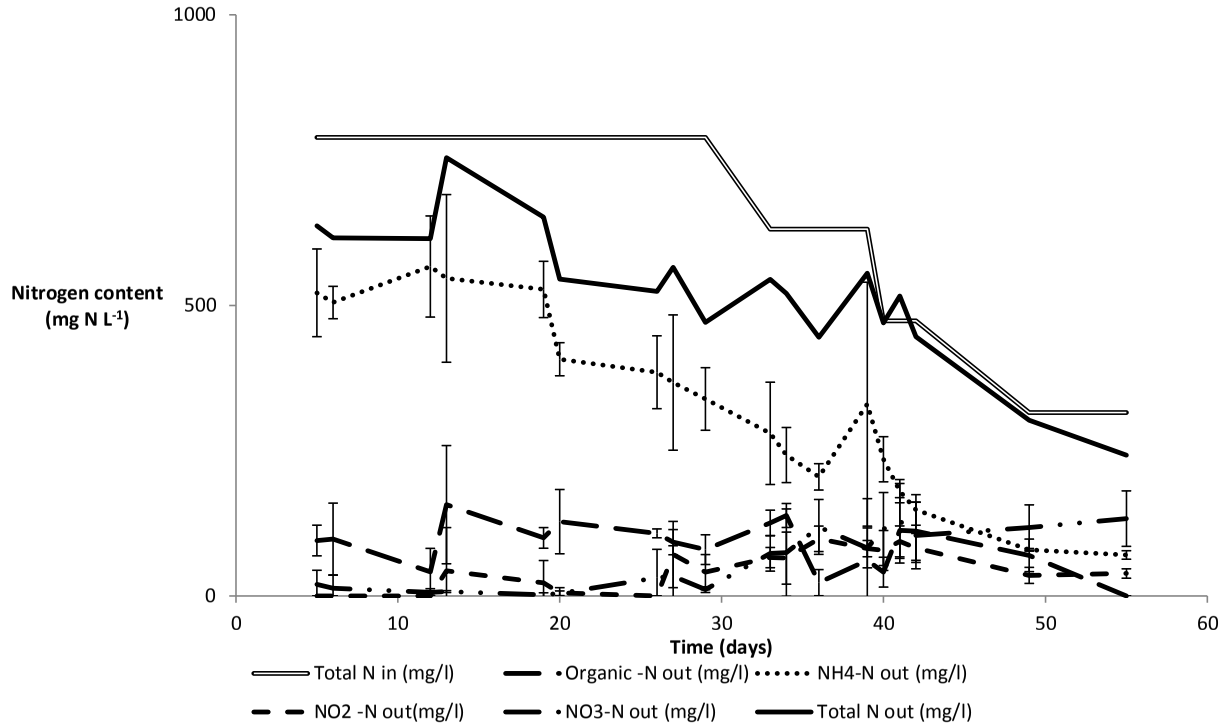
Parameters	Organic nutrient solution (OF)	Inorganic nutrient solution (IF)
pH(H_2O)	3.3	6.8
Carbon content (mg C L^{-1})	17.5	0
C/N ratio	2.18	0
Conductivity (µS cm^{-1})	955	2012
Organic-N (mg N L^{-1*})	186.0	0
Urea-N (mg N L^{-1})	603.0	0
Total Ammonia- N (mg N L^{-1})	12.4	16.6
Nitrate-N (mg N L^{-1})	1.6	288.7
Total-nitrogen (mg N L^{-1})	803.0	305.4
Phosphorous (mg P L^{-1})	90.8	55.3
Potassium (mg K L^{-1})	112.9	371.9
Calcium (mg Ca L^{-1})	30.0	316.2
Magnesium (mg Mg L^{-1})	14.8	119.1
Sulphur (mg S L^{-1})	17.2	221.3
Sodium (mg Na L^{-1})	6.9	0
Chloride (mg Cl L^{-1})	98.6	0



Supplementary figure 3.1: Evolution of the nitrate content in the effluent in an organic and mineral growing with an organic and inorganic fertigation system. GBIF: organic growing medium with organic fertilizer (100% organic derived nitrogen), RWIF: mineral growing medium with inorganic fertilizer GBOF: organic growing medium with organic fertilizer (100% organic derived nitrogen) and RWOF: mineral growing medium with organic fertilizer



Supplementary figure 3.2: Evolution of the DO levels in the effluent in an organic and mineral growing media with an organic and inorganic fertigation system. GBIF: organic growing medium with organic fertilizer (100% organic derived nitrogen), RWIF: mineral growing medium with inorganic fertilizer GBOF: organic growing medium with organic fertilizer (100% organic derived nitrogen) and RWOF: mineral growing medium with organic fertilizer



Supplementary figure 3.3: N mineralization in an organic growing medium with an organic derived nitrogen source in function of time. The used organic nitrogen source is (Total N in) is 100% organic derived nitrogen.

CHAPTER 4: THE USE OF MICROALGAE AS A HIGH-VALUE ORGANIC SLOW-RELEASE FERTILIZER RESULTS IN TOMATOES WITH INCREASED CAROTENOID AND SUGAR LEVELS

This chapter has been redrafted after:

Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., & De Gelder, L. (2015). The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. *Journal of Applied Phycology*, 1-11.

Abstract

The heightened awareness concerning environmental preservation, resource scarcity, food safety and nutrition, has engendered the need for a more sustainable and resource-efficient agricultural production system. In this context, microalgae offer the potential to recover nutrients from waste streams and subsequently use the microalgae biomass as a sustainable slow-release fertilizer. We hypothesized, that novel recovered fertilizers, such as microalgae and organic fertilizer could replace conventional fertilizers resulting in a comparable plant performance (yield and quality). The aim of this study was to assess microalgae bacterial flocs treating aquaculture wastewater and marine microalgae as organic slow-release fertilizers for tomato cultivation. Comparable plant growth was observed using microalgae and commercial organic fertilizer treatments. Furthermore, the microalgae fertilizers improved the fruit quality through an increase in sugar and carotenoid content, although a lower tomato yield was obtained. An economic evaluation indicates the economic feasibility of the microalgae-based fertilizers. Further research is required to optimize the microalgae-based fertilizer composition.

1. Introduction

In the light of the growing global population, rising resource scarcity and environmental preservation, the transition towards a more sustainable food production system is becoming increasingly important (Sutton et al. 2013). The implementation of innovative nutrient recycling technologies, green fertilizers and advanced cultivation practices is needed to further increase agricultural outputs, to improve nutrient use efficiencies and reduce nutrient losses. Modern glasshouse horticulture is characterized by high crop yields and a stable year-round supply of high quality fruits and vegetables. This high crop output is often accompanied by the intensive and unsustainable use of biocides, inorganic fertilizers and soilless culture techniques (Vox et al. 2010). The heightened social awareness concerning environmental preservation, food safety and nutrition, has engendered an increasing consumer interest in sustainable and organic products (Kondo et al. 2010). Conventional glasshouse practices are therefore being reassessed to include the use of high quality organic fertilizers and growing media that enable the production of sustainable, safe and nutritional fruits and vegetables. Indeed **[Chapter 2] showed** that **Unclassified *Nitrospira*** were significantly associated with GB and that the **different constituents and the blend showed specific functionalities, i.e. ammonia and nitrite oxidation rates**.

The cultivation of microalgae has been studied extensively within the field of biotechnology. Microalgae are a rich source of carbohydrates, lipids, proteins, pigments and vitamins and are therefore a valuable feedstock for cosmetics, biofuels and food and feed supplements (Spolaore et al. 2006). Moreover, they allow for the efficient recovery of the nitrogen (N) and phosphorus (P) present in wastewaters by concentrating these nutrients in algal biomass (Cai et al. 2013). The valorization of wastewater-grown microalgae for food or feed purposes is, however, in general legally restricted (Van Den Hende et al. 2014b). Although much less studied, the microalgae biomass can instead be employed as an organic slow-release fertilizer (Mulbry et al. 2007; Mulbry et al. 2005).

As an organic fertilizer, microalgae have the potential to prevent nutrient losses through a gradual release of N, P and potassium (K), which is attuned to the plant requirements (Mulbry et al. 2007). Besides macronutrients, phototrophic micro-organisms also contain trace elements and plant growth-promoting substances such as phytohormones, vitamins, carotenoids, amino acids and antifungal substances (Spolaore et al. 2006). The beneficial characteristics of phototrophic biomass as a bio fertilizer, i.e. bioactive growth-promoting amendments, have already been demonstrated for cyanobacteria, anoxygenic phototrophic bacteria and macro algae (Kobayashi and Kobayashi 1995; Kumari et al. 2011; Tripathi et al. 2008). The application of these bio fertilizers stimulated plant growth and increased crop yields. In addition, the cultivation of persimmon and tomato fruits resulted in improved fruit quality, through higher sugar and carotenoid concentrations in the fruits (Kobayashi and Kobayashi 1995; Kumari et al. 2011). This indicates that nutrients recovered through microalgae cultivation can be recycled as microalgae-based fertilizers to improve the quality and market value of high-value fruits such as tomatoes.

We hypothesized, that novel recovered fertilizers, such as microalgae and organic fertilizer could replace conventional fertilizers resulting in a comparable plant performance (yield and quality). Two types of microalgae biomass were evaluated: microalgae bacterial flocs (MaB-flocs) from a raceway pond treating aquaculture wastewater (Van Den Hende et al. 2014a) and a marine culture of *Nannochloropsis* sp. cultivated on a landfill site. In a first stage, the microalgae biomass composition and N and P mineralization rates were determined. Subsequently, a tomato cultivation experiment in

a glasshouse was performed, wherein the fertilizer potential of both types of microalgae biomass was compared with conventional inorganic and organic horticulture fertilizer systems. The growth rate of the tomato plants and the tomato yield were assessed for each fertilizer treatment, as well as the composition of the leaves and the water and sugar and carotenoid concentrations in the tomato fruits. Afterwards, the economic feasibility of microalgae fertilizers was determined through a comparison with conventional inorganic and organic glasshouse cultivation systems. To the authors' knowledge, this study is the first to assess the application of microalgae biomass as fertilizers for glasshouse tomato production.

2. Materials and methods

2.1 Microalgal biomass

Microalgal bacterial flocs (MaB-flocs) were harvested during August 2013 from an outdoor raceway pond (28 m²). The raceway pond was operated as a sequencing batch reactor and treated wastewater from a recirculating freshwater aquaculture system for the cultivation of pikeperch (*Sander lucioperca* L.; Inagro, Roeselare, Belgium) (Van Den Hende et al. 2014a).

Table 4.1: Elemental composition of the dried MaB-floc and Nannochloropsis biomass

	MaB-flocs	***Nannochloropsis***
Ash (weight-%)	67.12	23.22
N (weight-%)	2.44	8.07
P (weight-%)	0.59	1.29
K (weight-%)	0.18	1.36
N:P:K	13.6:3.3:1	6:1:1
Ca (weight-%)	20.40	0.20
Mg (weight-%)	0.21	0.40
S (weight-%)	0.24	0.05
Na (weight-%)	0.03	1.34
Fe (mg kg^{-1} dried biomass)	328	143
Cu (mg kg^{-1} dried biomass)	7.86	11.8
Zn (mg kg^{-1} dried biomass)	143	66.7
B (mg kg^{-1} dried biomass)	5.65	1.16
Mn (mg kg^{-1} dried biomass)	42.5	113

Upon gravitational settling, MaB-flocs were dewatered in filter bags (150-250 µm pore size; Lampe, Belgium) by a hydropress (4 bar; Enotecnica Pillan, Italy). MaB-flocs were dominated by filamentous microalgae (*Ulothrix* sp. and *Klebsormidium* sp.) (Van Den Hende et al. 2014a). *Nannochloropsis*

oculata was obtained from Proviron (Hemiksem, Belgium) and was produced on a 700 m² algae pilot plant, located on a landfill site (Hooge Maey, Antwerp, Belgium).

The microalgae were cultivated in outdoor closed flat panel photobioreactors (25 m²) in modified Guillard's f/2 marine medium, using flue gas CO_2 and residual heat from the landfill biogas cogeneration installation (Proviron, personal communication). After cultivation, the algae were concentrated using microfiltration followed by centrifugation. The dewatered MaB-flocs and *Nannochloropsis* biomass were pasteurized and dried at 70°C for 14 hours and subsequently pulverized. The elemental biomass compositions are described in Table 4.1. Heavy metal content of the MAB flocs, Nannochloropsis and the organic fertilizer were not determined.

2.2 Organic growing medium

The organic growing medium (GB, Peltracom, Gent, Belgium) used during the mineralization and tomato cultivation experiments was a nutrient-poor mixture of sod peat (40% v/v), Irish peat (40% v/v) and coconut fiber (20% v/v). Calcium and magnesium carbonate (Dolokal Extra PG, Ankerpoort, The Netherlands) were added at 2.5 kg m^{-3} to reach an optimal pH (H_2O) of 5.5. Chemical characteristics of the growing medium pH(H_2O), electrical conductivity (EC), ammonium, nitrate, phosphorus, potassium, calcium, magnesium, sulphate, sodium, chloride, iron and manganese) were measured according to Gabriels et al. (1998a). Potassium, phosphorus, calcium, magnesium, iron and manganese were extracted (1:5 vol/vol) in ammonium acetate and measured with ICP. The electrical conductivity (EC), pH(H_2O), ammonium (NH_4^+), nitrate (NO_3^-), sulphate (SO_4^{2-}) and sodium (Na) were measured in a 1:5 v/v water extract according to EN 13038, EN 13037 and EN 13652, respectively. Nitrate was measured with an IC ion chromatograph. Ammonium was measured by steam distillation. Physical characteristics of the growing media were measured according to EN 13041 (DIN 2012). The physical and chemical characteristics of the growing medium can be found in Supplementary table 4.1. Each treatment was analyzed in triplicate (n=3).

2.3 Microalgal N and P mineralization rate analysis

The N and P mineralization rates of the dried MaB-flocs and *Nannochloropsis* biomass were determined according to Chaves et al. (2004). The organic growing medium was mixed with the microalgae biomass to obtain a final concentration of 555 mg biomass-TN L^{-1} of growing medium. The microalgae-growing medium mixtures were transferred to PVC tubes (diameter = 4.63 cm; filling height = 12 cm; dry bulk density = 140 kg m^{-3}) and distilled water was added to obtain a volumetric water content, that was equal to 80% of the water content at a matric potential of -1 kPa. The tubes were incubated at 20 °C and samples were taken after 0, 7, 14, 21, 35, 67 and 95 days. At each sampling time, tubes were collected in quadruplicate (n=4). Growing medium extractions using distilled water (10:1 water:g growing medium) were performed for the determination of pH(H_2O), EC, total ammonium nitrogen (TAN), nitrite, nitrate, phosphate and sulphate. Mehlich-3 extractions (10:1 extractant:g growing medium) were performed to determine the concentration of plant-extractable P (Mehlich 1984).

2.4 Tomato cultivation set-up

Solanum lycopersicum cv. 'Maxifort' were sown on the 29th of October 2013 and *Solanum lycopersicon* cv. 'Merlice' were grafted on the stems on the 15th of November 2013. The plants were grown on slabs

filled with non-sterilized organic growing medium (Peltracom, Belgium). The cultivation experiment consisted of four fertilizer treatments: (1) a liquid inorganic fertilizer, (2) solid organic fertilizer, (3) MaB-floc fertilizer and (4) *Nannochloropsis* fertilizer (Figure 4.1).

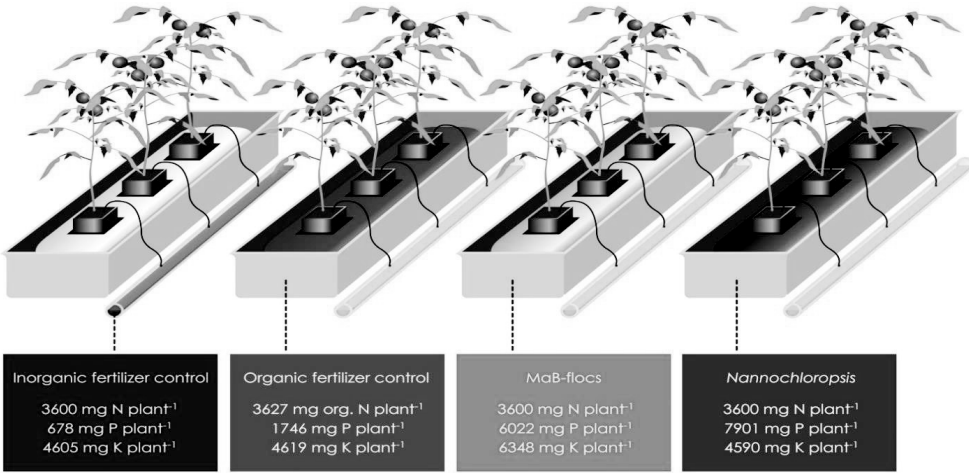


Figure 4.1: Overview of the tomato cultivation set-up. The set-up consists of a liquid inorganic fertilizer control treatment, a solid organic fertilizer control treatment, a MaB-floc fertilizer treatment and Nannochloropsis fertilizer treatment.

The fertilizer demand of the plants was calculated according to commercial glasshouse tomato practices (Sonneveld and Voogt 2009b). For the inorganic fertilizer control treatment a standard fertilizer solution suitable for the growth of tomato plants (Sonneveld and Voogt 2009b) was dosed with a standard drip irrigation system. The organic fertilizer control treatment consisted of a blend of two commercial solid fertilizers SF1 (66% of N demand) and SF2 (33% of N demand) (Frayssinet, France), which were blended in the growing medium prior to the start of the cultivation experiment. SF1 was characterized by a nutrient content of 4% N, 2.2% P and 5% K and a mineralization coefficient of 90% mineralization after three months. SF2 had a nutrient content of 8% N, 2.2% P and 5% K and a mineralization coefficient of 80% after three months. The nutrient demand of the plants for a cultivation period of three months was added to the growing medium prior to the start of the experiment (Table 4.2).

For both microalgae treatments the microalgae fertilizer was blended with the organic growing medium, considering an N mineralization rate of 33% after three months. The organic and microalgae fertilizer treatments were amended with kali vinasses (38% K_2O; Rendapart, Belgium) and Patentkali® (30% K_2O, 10% MgO and 42% SO_3; Pillaert Meststoffen, Belgium), respectively, to obtain a comparable macronutrient ratio in the growing.

For each fertilizer treatment, three slabs (1.0 m × 0.2 m × 0.085 m) with organic growing medium were used. Five slabs were used per treatment, however only three slabs were used for the experiment to avoid confounding effect. Three plants were grown per slab, with an interspacing of 50 cm between plants to obtain nine plant replicates (n=9) in combination with 3 slabs. The fertilized slabs were

incubated for one week prior to the plant cultivation experiment to initiate microbial activity and mineralization in the growing medium. The 134 days-old tomato plants were planted on top of the different growing media on the 12th of March 2014. A drip irrigation system was used for a controlled dosage of rainwater. The amount of irrigation water was the same for each treatment and was set to achieve a minimum drain to prevent nutrient losses through leaching, although in commercial growing systems generally a drain of 30% is applied. The drainage was not reused, hence plants were growing in an open soilless culture system. The glasshouse was equipped with an automated ventilation and heating system and accommodated with movable screens for shading (ILVO, Melle, Belgium). Temperature was maintained at 17 °C during the night and above 22 °C during the day. Additional artificial lighting was provided from 8 am to 9 pm at 50 µmol PAR m^{-2} s^{-1}.

*Table 4.2: Composition of the different fertilized growing media at the start of the tomato cultivation experiment (n=3): "-"= not present. * Chemical analysis are expressed as mg L^{-1} growing medium*

parameters	Inorganic fertilizer	Organic fertilizer	MaB-flocs	*Nannochloropsis*
pH(H_2O)	5.79 ± 0.04	5.77 ± 0.01	7.23 ± 0.05	5.13 ± 0.12
EC (µS cm^{-1})	58 ± 4	462 ± 112	205 ± 44	875 ± 356
NO_3^--N (mg L^{-1*})	6.78 ± 5.14	-	-	-
NH_4^+-N (mg L^{-1})	1.14 ± 0.13	55.63 ± 24.21	47 ± 4	6.22 ± 3.52
P (mg L^{-1})	11.05 ± 0.75	38.03 ± 7.70	268 ± 66	239 ± 68
K^+ (mg L^{-1})	56 ± 3	1006 ± 288	189 ± 40	784 ± 282
Ca^{2+} (mg L^{-1})	589 ± 53	578 ± 55	15634 ± 2587	636 ± 136
Mg^{2+} (mg L^{-1})	132 ± 9	193 ± 28	199 ± 22	277 ± 55
SO_4^{2-} (mg L^{-1})	129 ± 12	888 ± 168	126 ± 86	1239 ± 635
Na^+ (mg L^{-1})	40 ± 2	167 ± 40	48 ± 5	360 ± 118
Cl^- (mg L^{-1})	85 ± 7	201 ± 42	66 ± 10	648 ± 223

Tomato plant growth was monitored by determining the plant height and diameter three times per week, just below the first leaf on the bottom of the plants. Side shoots were removed according to commercial practice while tomato trusses were left unpruned. Pollination of the flowers was done by hand. Tomato leaves were harvested on regular time intervals and analyzed for their length, weight, elemental composition, dry matter and ash content. Growing media samples were taken on regular time intervals and a water extraction was performed to determine the pH(H_2O), EC and elemental composition. Tomato fruits were harvested and the fresh weight, dry matter content, sugars and carotenoid concentrations were determined.

2.5 Analytical techniques

Elemental growing medium and biomass compositions were determined using ICP-OES (Vista-MPX, Varian, Australia) as described by Greenberg et al. (1992). Nitrate, nitrite and phosphate were analyzed after sample filtration using anion chromatography (Metrohm 761 Compact IC, Switzerland).

Ammonium (Nessler method), total Kjeldahl nitrogen (TKN), and total phosphorus (TP) (molybdate:vanadium method) were determined according to standard methods (Greenberg et al. 1992). The chlorophyll and carotenoid content of the tomato fruits were determined according to (Lichtenthaler 1987). Sugars were extracted according to De Swaef et al. (2012). Glucose and fructose were determined using KPAEC-PAD (ICS 3000, Dionex, USA) with a 3x150mm CarboPac PA20 analytical column.

2.6 Statistical analysis

A non-parametric non-paired Kruskal-Wallis test was performed and combined with an all-pairwise multiple comparison Dunn's test to compare tomato yields and fruit quality parameters (Prism 5.0, GrapkPad Software, USA). Homogeneity of variances was verified with Levene's test. For the physicochemical characteristics of the leaves and the growing medium a one way ANOVA repeated measures analysis of variance was conducted to evaluate the null hypothesis that there is no significant difference between the treatments. Sphericity and normality of dependent variables were assessed using a Mauchly test and Shapiro-Wilk test, respectively. In the case that sphericity was not obtained corrections were performed according to Greenhouse-Geisser. P-values were adjusted with the Bonferroni confidence interval. Pairwise differences between treatments were considered significant at $P<0.05$.

3. Results and discussion

3.1 Mineralization of the microalgae biomass

A batch incubation study was performed to determine the N and P mineralization rate for the two types of microalgae biomass. The MaB-flocs and *Nannochloropsis* had an initial N availability of 7% and 5%, respectively (Figure 4.2A).

After 21 days, 11% and 16% of the biomass-N was mineralized, respectively. A final plant-available N fraction of 25% and 31% was obtained after 95 days for the MaB-flocs and *Nannochloropsis* biomass, respectively. An initial plant-extractable P availability of 53% was obtained for both biomass types. Also, an initial ortho-phosphate availability of 11% P for MaB-flocs and 7% P for *Nannochloropsis* was measured (Figure 4.2B). These values did not increase significantly during the incubation period. Both algal biomass types showed a similar mineralization profile. However, the N mineralization rates are lower than previously described, as algal biomass grown on manure effluents showed a N availability of 25-33% after 21 days and a maximum N availability of 41% after 63 days (Mulbry et al. 2007; Mulbry et al. 2005). The mineralization rates are nonetheless within the range of commercially available slow-release fertilizers which are applied in organic horticulture (Stadler et al. 2006a). The growing medium environment controls the release of N and P from organic nutrient sources. Discrepancies in mineralization rates may therefore be explained by differences in growing media microbial community composition, water content, temperature and pH during the incubations (Agehara and Warncke 2005). The lower microbial activity in our study is reflected in the low nitrification activity obtained during the mineralization test, as less than 2% of the available ammonium nitrogen was oxidized to nitrite or nitrate.

Furthermore, plant growth and root activity support the release of nutrients from fertilizers through the excretion of mobilizing substances such as organic acids (Ahmed et al. 2015). The prediction of the

plant available nutrients is therefore more accurately described by plant uptake than by chemical extraction. In glasshouse production systems, where irrigation and temperature can be controlled, fertilizer management that considers both the nutrient source and growing medium environment can further improve the effectiveness of the chosen organic fertilizer materials.

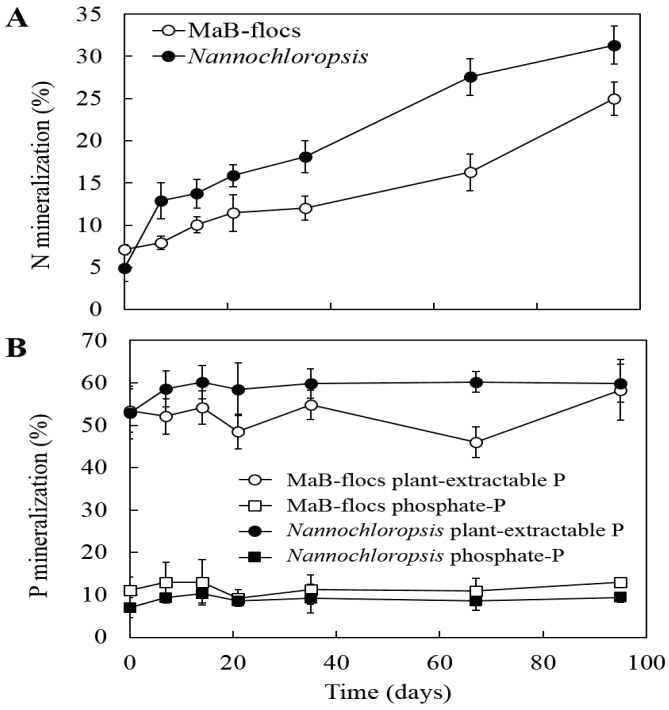


Figure 4.2: N (A) and P (B) mineralization profile of dried MaB-floc and Nannochloropsis biomass (n=4). Error bars indicate standard deviation.

3.2 Effect of microalgae biomass on tomato plant performance

3.2.1 Tomato plant growth

The high concentration of calcite ($CaCO_3$) precipitates present in the MaB-flocs (Van Den Hende et al. 2014a) resulted in an increase in the calcium concentration and pH(H_2O) of the growing medium compared to the control treatments. The marine *Nannochloropsis* biomass resulted in elevated sodium, sulphate and chloride concentrations and an increased EC of the growing medium. The ammonium concentration of the *Nannochloropsis* and the MaB-flocs treatment were comparable to the organic control treatment, but higher than the inorganic control. For both microalgae fertilizers, a higher phosphorus concentration was obtained in the growing medium.

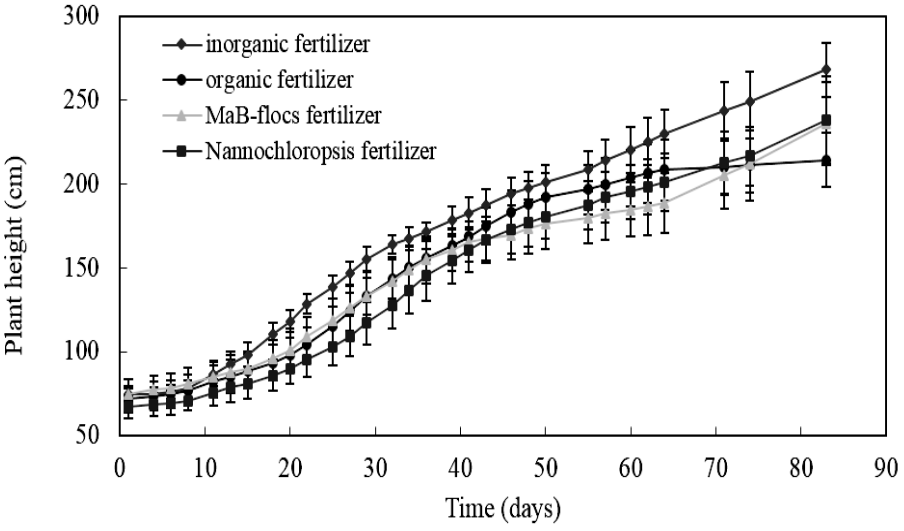


Figure 4.3: Growth curve of the tomato plants (n=9) for the different fertilizer treatments. Error bars indicate standard deviation

There was no significant difference between the growth rate of plants grown on the algal fertilizers and the organic fertilizer. Compared to the organic and algal fertilizers, the conventional inorganic growing system revealed a faster initial plant growth (Figure 4.3), which is attributed to the readily available inorganic nutrients and the presence of nitrate as the predominant nitrogen source (Sonneveld and Voogt 2009b). The faster plant growth is confirmed by the higher mean plant length (162.5 ± 3.8 cm), i.e. the average plant length over the duration of the cultivation experiment, in comparison with the organic treatment (145.6 ± 3.8 cm), MaB-flocs treatment (143.2 ± 17 cm) and *Nannochloropsis* treatment (139.2 ± 3.8 cm) (Supplementary table 4.2). Nevertheless, there was no significant difference in the final plant height for the organic fertilizer, MaB-flocs and *Nannochloropsis* treatment (Figure 4.3). Growth suppression due to ammonia toxicity therefore did not occur. In addition, no significant difference was observed in mean stem diameter and the number of trusses among the different treatments (Supplementary table 4.2).

Leaf analysis showed significantly higher nitrogen content for the algal fertilizer treatments, which demonstrates the good nitrogen fertilizer properties of the algal fertilizers (Table 4.3). Additionally, a lower ash content of the tomato plant leaves was observed for both algal fertilizer treatments throughout the experiment. The MaB-floc fertilizer resulted in a significantly lower magnesium and potassium content of the leaves (Table 4.3).

This deficiency was visually noticeable as interveinal chlorosis and can be attributed to the relatively high calcium concentration in the algal biomass, which can inhibit the uptake of magnesium and potassium (Jakobsen 1993). A minor magnesium deficiency is nevertheless common in glasshouse horticulture and rarely results in yield reduction. Although the high pH(H_2O) of the MaB-floc fertilizer could lower the plant availability of micronutrients, no symptoms of micronutrient deficiencies were observed.

Table 4.3: Leaf characteristics for the different fertilizer treatments during the experiment[1]

	Inorganic fertilizer	Organic fertilizer	MaB-flocs	*Nannochloropsis*
Leaf length (cm)	40.7±1.1	40.6±1,1	37.8±1.1	42.2±1.1
Leaf fresh weight (g)	20.7±1.2	19.8±1.2	19.3±1.2	23.5±1.2
Leaf dry weight (%)	18.2±0.6 [a]	19.0±0.6 [a]	20.8±0.6 [a]	16.3±0.6 [b]
Ash (weight-% DM)	17.78±0.38 [b]	16.11±0.38 [b]	13.56±0.38 [a]	12.64±0.38 [a]
N (weight-% DM)	2.83±0.11 [c]	3.84±0.11 [b]	4.7±0.11 [ab]	5.05±0.11 [a]
P (weight-% DM)	0.42±0.26 [b]	0.72±0.26 [a]	0.79±0.26 [a]	0.71±0.26 [a]
K (weight-% DM)	46.00±3.96	30.95±3.96	21.46±3.96	37.50±3.96
Ca (weight-% DM)	27.21±1.09 [a]	21.56±1.09 [bc]	25.96±1.09 [ab]	12.54±1.09 [a]
Mg (weight-% DM)	6.2±0.2 [b]	7.2±0.2 [a]	5.2±0.2 [b]	7.1±0.2 [a]
Zn (weight-% DM)	0.040±0.004	0.039±0.004	0.039±0.004	0.034±0.004
Mn (weight-% DM)	0.15±0.16 [a]	0.12±0.16 [ab]	0.11±0.16 [bc]	0.05±0.16 [c]
Fe (weight-% DM)	0.064±0.004	0.053±0.004	0.050±0.004	0.055±0.004
Cu (weight-% DM)	0.014±0.005	0.005±0.005	0.003±0.005	0.002±0.005
B (weight-% DM)	0.02±0.02 [b]	0.32±0.02 [a]	0.29±0.02 [a]	0.01±0.02 [b]

[1] Mean values and standard error of means over the different treatments are displayed of four sampling points (day 22, 36, 60 and 90). Bonferroni-corrected p-values are calculated for the effect of fertilization strategy and time on leaf length, fresh and dry weight and chemical characteristics of leaves in tomato plants (n =3) per treatment and per time point. Factors, which do not share superscripts, are significantly different from each other ($P < 0.05$).

The *Nannochloropsis* treatment resulted in lower calcium concentrations in the tomato leaves. Calcium deficiency is often the result of water stress and can therefore be attributed to the elevated salt concentrations of the marine microalgae biomass, since absolute calcium levels did not differ from the controls (Table 4.4). Also, the high magnesium content can induce calcium deficiency, as the same plant absorption sites are shared for the uptake of calcium, magnesium, and potassium (Papadopoulos 1991). No significant difference in iron (Fe), copper (Cu) and zinc (Zn) content was observed among treatments. These results show that with regard to plant growth, both the MaB-flocs and marine *Nannochloropsis* biomass can replace conventional inorganic and organic fertilizers.

*Table 4.4: Characteristics of the organic growing medium for the different fertilizer treatments during the experiment. Mean values and standard error of the means over the different treatments are displayed of five sampling points (day 1, 22, 36, 62 and 90). Bonferroni-corrected p-values are calculated for the effect of fertigation strategy and time on the chemical characteristics of the organic growing media in tomato plants (n=3). Per treatment and per time point. Factors which do not share superscripts are significant different from each other (P=0.05). * Chemical analysis are expressed as mg L^{-1} growing medium*

	Inorganic fertilizer	Organic fertilizer	MaB-flocs	*Nannochloropsis*
pH(H_2O)	5.73±0.07 ^{c}	5.58±0.07 ^{c}	7.25±0.07 ^{a}	6.11±0.07 ^{b}
EC (µS cm^{-1})	86.3±50.8 ^{b}	504.1±50.8 ^{a}	243.3±50.8 ^{b}	657.9±50.8 ^{a}
NO_2^--N (mg L^{-1*})	0.16±0.08 ^{b}	0.22±0.08 ^{ab}	0.56±0.08 ^{a}	0.18±0.08 ^{b}
NO_3^--N (mg L^{-1})	7.2±0.5 ^{a}	1.9±0.5 ^{c}	5.3±0.5 ^{ab}	3.7±0.5 ^{bc}
NH_4^+-N (mg L^{-1})	2.8±1.6 ^{b}	34.4±1.6 ^{a}	36.3±1.6 ^{a}	37.0±1.6 ^{a}
P (mg L^{-1})	10.5±10.8 ^{b}	43.6±10.8 ^{b}	243.7±10.8 ^{a}	167.0±10.8 ^{a}
K^+ (mg L^{-1})	25.2±37.6 ^{c}	302.8±37.6 ^{ab}	140.5±37.6 ^{bc}	465.7±37.6 ^{a}
Ca^{2+} (mg L^{-1})	678±1113 ^{b}	665±1928.2 ^{b}	12986±1113 ^{a}	603.7±1113 ^{b}
Mg^{2+} (mg L^{-1})	184.5±28.8 ^{b}	232.3±20.4 ^{ab}	197.5±16.6 ^{b}	319.8±20.4 ^{a}
SO_4^{2-} (mg L^{-1})	202.7±114.2 ^{b}	1131.1±114.2 ^{a}	180.5±114.2 ^{b}	983.4±114.2 ^{a}
Na^+ (mg L^{-1})	45.5±18.5 ^{b}	122.5±13.1 ^{b}	73.0±10.7 ^{b}	256.5±13.1 ^{a}
Cl^- (mg L^{-1})	32.4±16.4 ^{b}	57.1±16.4 ^{b}	41.4±16.4 ^{b}	258.2±16.4 ^{a}

[1] Mean values and standard error of means over the different treatments are displayed of five sampling points (day 1, 22, 36, 62 and 90). Bonferroni-corrected p-values are calculated for the effect of fertilization strategy and time on the chemical characteristics of organic growing media in tomato plants (n =3) per treatment and per time point. Factors which do not share superscripts are significantly different from each other (*P*=0.05).

3.2.2 Tomato fruit yield

There was no significant difference in the number of fruits between treatments. A lower tomato yield was nevertheless obtained for the algal treatments compared to the inorganic and organic systems (Figure 4.4). In addition, a higher incidence of blossom-end rot was observed for both the organic and algal fertilizers. The fresh weight of the red tomatoes was significantly higher for the conventional inorganic fertilizer treatment, but no difference was observed among the organic and algal treatments (Figure 4.5A). The lower marketable yield and fruit size obtained for the organic and algal fertilizer treatments can be explained by different phenomena. Lower tomato yields have been commonly observed under salt stress conditions (Magán et al. 2008). The lower yields in this study can therefore be attributed to the osmotic pressure caused by an increased salinity of the organic and algal fertilizers, which limits the water flux to the developing fruits. Also differences in nitrogen availability can impose stress, as plants grown on ammonium tend to grow smaller fruits and are more affected by blossom-

end-rot than plants grown on nitrate (Borgognone et al. 2013). Blossom end rot is often connected with high ammonium concentration in combination with increased Cl^-, i.e. the Nannochloropsis treatment and SO_4^{2-} concentrations with the Nannochloropsis and the organic fertilizer treatment. This is in agreement with other studies that showed a higher BER incidence in combination with higher ammonium concentrations (Heeb et al. 2005a; Kinet and Peet 1997; Pilbeam and Kirkby 1992). Ammonium resembles potassium (K^+) in terms of ionic size and size of the hydration shell, thus, ammonium ions are able to pass through K^+-channels.

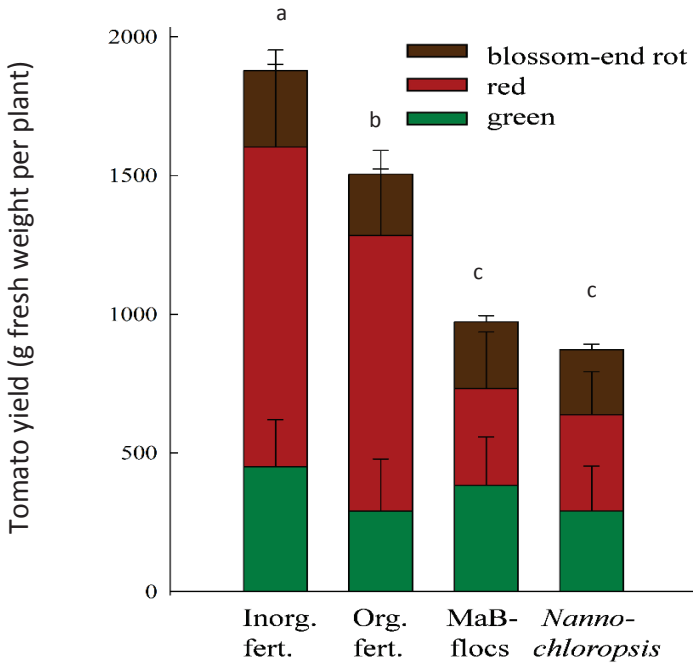


Figure 4.4: Tomato yield per plant (n=9) for each fertilizer treatment subdivided in the weight of green, red and blossom-end rot-affected tomatoes per plant. Error bars indicate standard deviation. Different letters (a, b, c) indicate significant differences between the treatments.

The low concentration of potassium (ten Hoopen et al. 2010) in ammonium fed plants leads to an upregulation of the K^+-channels to boost the potassium (K^+) uptake, potentially resulting even in a higher ammonium influx though these channels. Next to low potassium concentrations, also the content of other essential cations, like calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the plant are decreased and increased levels of sulphate (SO_4^{2-}), chloride (Cl^-), and phosphate (PO_4^{3-}) were found (Britto and Kronzucker 2002). These adverse effects of an increased supply of ammonium as a nitrogen source are also associated with a lower calcium and magnesium uptake, which was observed in the elemental leaf composition for the *Nannochloropsis* and MaB-floc fertilizers (Table 4.3). The lower yield in combination with the Mab flocs indicate that other factors than the osmotic pressure and differences in nitrogen availability might play an essential role. The pH(H_2O) of the growing medium and the especially the rhizosphere plays an important role for the uptake of micronutrients. Indeed the

pH(H_2O) of the growing medium with the Mab flocs has an increased pH(H_2O), which might cause a decreased uptake of Fe, Mn, Zn and Cu (Sonneveld and Voogt 2009b).

3.2.3 Tomato quality

Tomato fruits grown on *Nannochloropsis* and MaB-flocs had a 34% and 20% higher dry weight content, respectively, compared to the inorganic fertilizer treatment; whereas no difference between the inorganic and organic fertilizer treatment was observed (Figure 4.5B). Furthermore, both algal fertilizers resulted in significantly higher sugar concentrations in the fruits (Figure 4.5C). The *Nannochloropsis*-grown tomato fruits had a glucose concentration that was 18% higher than the organic and 33% higher than the inorganic fertilizer treatment. The MaB-floc fertilizer resulted in a 23% higher glucose concentration compared to the inorganic fertilizer. For the *Nannochloropsis*-grown tomatoes, a 21% higher fructose concentration was observed compared to those grown on inorganic fertilizers. The higher sugar concentrations observed confirm the importance of reduced nitrogen forms such as ammonium or organic nitrogen to improve the tomato fruit taste (Heeb et al. 2005b).

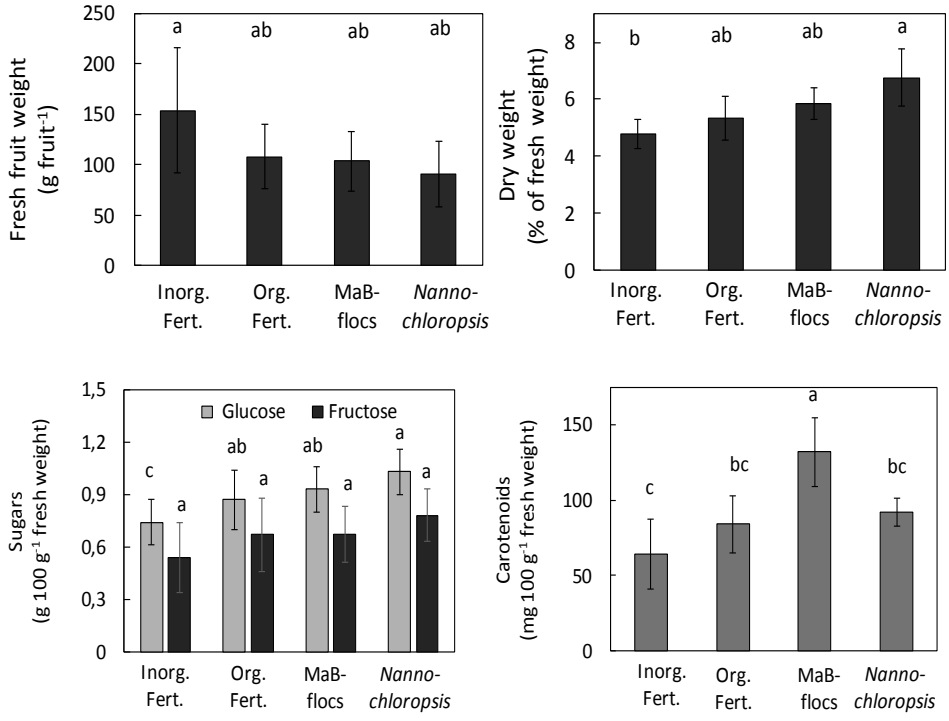


Figure 4.5: Fresh weight (A), dry weight (B), sugar (C) and carotenoid (D) content of red tomato fruits (n=36) for the different fertilizer treatments. Error bars indicate standard deviation. Significant differences amongst fertilizer strategies are indicated with a different letter.

The most remarkable improvement in fruit quality was observed for the carotenoid content (Figure 4.5D). Tomatoes grown on MaB-flocs contained 70% more carotenoids than in the inorganic fertilizer treatment and 44% more than in the organic fertilizer treatment. The effect of the *Nannochloropsis*

fertilizer was less profound, but still a 36% higher carotenoid content was obtained compared to the inorganic fertilizer treatment. These observations confirm the stimulating effect of phototrophic biomass on the sugar and carotenoid content of fruits (Kobayashi and Kobayashi 1995; Kumari et al. 2011). Moreover, it is in line with other findings, in which the inclusion of MaB-flocs as an aquaculture fodder supplement resulted in an increased red pigmentation of white Pacific shrimps (Van Den Hende et al. 2014b).

3.2.4 Potential of microalgae fertilizers in glasshouse horticulture

Globally, tomato is one of the most produced vegetables, ranking second after potato (Kumari et al. 2011). This illustrates the economic and nutritional importance of this crop. The increased sugar and carotenoid concentrations obtained with the algal fertilizers indicate the potential of microalgae-based fertilizers to increase the quality and economic value of tomato fruits. Carotenoids play an important role in many plants during photosynthesis, the protection against photo oxidative stress and attraction of insects. The amendment of microalgae biomass can therefore also have beneficial effects for other high-value plants. Comparable to tomatoes, microalgae fertilizers can improve the value of peppers (*Capsicum annuum*), while they can also be implemented in flower cultivation, as carotenoids induce the typical yellow and orange color in for instance roses (Lachman et al. 2001)

Nevertheless, the difference in fruit yield compared to the conventional horticulture fertilizers indicates that a more optimal fertilizer mixture is required to combine high quality fruits with satisfactory yields. The importance of nitrate to stimulate plant growth and fruit yield suggests that conventional fertilizers should be employed as the main source of macronutrients, while the addition of microalgae biomass could improve the market value of the products. Although further research is required to assess the optimal amount of microalgae to be amended to the fertilizers, previous findings related to the application of phototrophic organisms as bio fertilizers suggest that the microalgae biomass can already have beneficial effects on crop output at a 10% concentration (Kumari et al. 2011; Tripathi et al. 2008). Microalgae-based fertilizers can therefore partake in the transition of conventional glasshouse practices towards an Integrated Plant Nutrition System (IPNS). This integrated cultivation concept combines and optimizes the use of inorganic, organic and bio fertilizers to sustain desired crop productivity with a minimal impact on the environment (Chen 2006). The beneficial effects obtained in this study demonstrate the potential of microalgae fertilizers to give a benefit within this sustainability framework. Also in organic horticulture, conventional organic fertilizers can be amended with microalgae biomass to improve fruit quality, while preserving the sustainable organic cultivation practices.

Microalgae-based fertilizers also offer advantages within a larger sustainability framework. In contrast to nutrient-rich waste streams such as manure, microalgae biomass can function as a stable, predictable, transportable and concentrated fertilizer product. This allows it to be introduced in modern glasshouse horticulture. Furthermore, the additional plant growth-promoting characteristics of the microalgae biomass demonstrate that nutrient recovery through microalgae cultivation give an added value compared to the direct application of waste streams on cropland (Mulbry et al. 2007). The production of microalgae biomass from waste streams could therefore transform waste nutrients into sustainable high-value fertilizers with commercial relevance in glasshouse horticulture systems. However, as sustainability comprises environmental, economic and social aspects, chemical food

safety hazards, such as the heavy metal content of the algal biomass, must be elucidated to see their real potential.

4. Conclusions

The use of advanced nutrient recycling technologies and green fertilizers is pivotal in the transition towards a more sustainable and resource-efficient food production system. This study demonstrates that microalgae biomass can be used as an organic slow-release fertilizer for tomato cultivation. The microalgae fertilizers improve the quality of the fruits produced through an increase in the sugar and carotenoid content of fruits. Further research is required to determine optimal fertilizer mixtures that produce high quality fruits with satisfactory yields. In this context, our economic evaluation supports the economic feasibility of microalgae-based fertilizers, in which conventional fertilizers are supplemented with recycled microalgae biomass.

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Supplementary information

*Supplementary table 4.1: Chemical and physical characteristics of the organic growing medium. OM= organic matter; DM=dry matter. * Chemical analysis are expressed as mg L^{-1} growing medium*

Parameter	Value	Parameter	Value
pH(H_2O)	5.70 ± 0.10	Ash content (weight -% of DM)	3.5 ± 0.6
Conductivity (µS cm^{-1})	90.63 ± 20.99	Available water content (m^3 m^{-3})	0.218 ±0.036
Organic N (mg L^{-1*})	0.32 ± 0.55	DM (weight -% of fresh product)	41.5 ± 0.6
NO_2^--N (mg L^{-1})	0.00 ± 0.00	Shrinkage (m^3 m^{-3})	0.21 ± 0.02
NO_3^--N (mg L^{-1})	6.78 ± 5.14	Air content at –1kPa matric potential (m^3 m^{-3})	0.33 ± 0.04
NH_4^+-N (mg L^{-1})	6.05 ± 0.51	OM (weight -% of DM)	96.5 ± 0.6
P (mg L^{-1})	8.51 ± 1.31	Dry bulk density (kg m^{-3})	143.5 ± 11.3
K^+ (mg L^{-1})	103.75 ± 31.43	Total pore space (m^3 m^{-3})	0.92 ± 0.08
Ca^{2+} (mg L^{-1})	174.5 ± 28.72	Gravimetric water content (weight-%)	58.5 ± 0.6
Mg^{2+} (mg L^{-1})	54.75 ± 5.46	Volumetric water content at -1 kPa matric potential (m^3 m^{-3})	0.593 ± 0.040
SO_4^{2-} (mg L^{-1})	13.85 ± 3.01	Volumetric water content at -10 kPa matric potential (m^3 m^{-3})	0.405 ± 0.034
Na^+ (mg L^{-1})	42.75 ± 9.16	Available volumetric water content between -1 kPa and -10 kPa) (m^3 m^{-3})	0.218 ± 0.036
Cl^- (mg L^{-1})	76.80 ± 24.43		
$Fe^{2+/3+}$ (mg L^{-1})	0.10 ± 0.03		
Mn^{2+} (mg L^{-1})	0.60 ± 0.11		

The ash content is the ash or mineral content on dry matter basis; the available water content is the difference in water content between -1 kPa and -10 kPa matric potential and equals the plant available water content; DM is the dry matter content; OM is the organic matter content; dry bulk density is the density of a dried sample at 103°C; the air volume is that part of the volume of a growing medium sample filled by air at a predefined suction of -1 kPa matric potential; the process of a growing medium contracting to a lesser volume when subject to water loss is called shrinkage and is the volume of the fully saturated growing medium minus the volume of the dried growing medium divided by the volume of the fully saturated growing medium; the gravimetric water content is the amount of water on fresh weight basis of the growing medium. Drying of a sample is done by placing the samples without altering the structure in an oven at 103°C to constant mass.

Supplementary table 4.2: Mean values and standard error of means over the different treatments are displayed for the plant length and stem diameter. Bonferroni -Significant-Difference (P=0.05) are calculated for the tomato plants (n=3) per treatment and per time point. Differences among treatments were compared using a repeated measures mixed model in SAS (version 9.4, SAS Institute, Cary, USA) with the different tomato cultivating systems as a fixed effect and time as random effect. Significant differences amongst fertilizer strategies are indicated with a different letter.

	Inorganic fertilizer	**Organic fertilizer**	**MaB-flocs**	***Nannochloropsis***
Plant length (cm)	162.5 ±3.8 [a]	145.6 ±3.8 [b]	143.2 ±3.8 [b]	139.2 ±3.8 [b]
Stem diameter (mm)	10.9 ±0.2	10.9 ±0.2	10.4 ±0.2	10.7 ±0.2

CHAPTER 5: SOILLESS CULTURE SYSTEMS IN COMBINATION WITH ORGANIC FERTILIZERS REQUIRE AN ADAPTED FERTIGATION STRATEGY TO NARROW THE YIELD GAP WITH CONVENTIONAL SYSTEMS

This chapter has been redrafted after:

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Abstract

Soilless horticultural systems have the advantage that oxygen and water required for a healthy plant growth are controlled, soil-borne pathogens can be circumvented and nutrient stress is avoided through the use of inorganic fertilizers. However, these conditions are more difficult to attain in soilless culture systems in combination with organic fertilizers. We hypothesized, that in soilless tomato cultivation systems, a gradual increase of the organic nitrogen supply rate will result in comparable yields compared to inorganic fertilizers. In this experiment, the nitrogen supply rate, the nitrogen source (organic and inorganic) were changed to explore the two following research questions for a tomato crop grown in an open soilless culture system in combination with mineral and organic growing media. (1) Does a step-wise increase of the inorganic nitrogen supply rate deliver enough nutrients to the plant resulting in comparable plant performance compared to conventional systems? (2) Does a step-wise increase of the organic nitrogen supply rate result in a comparable plant performance compared to a conventional system? The nitrogen, phosphorous, potassium, calcium, magnesium, sulphate, sodium and chloride dynamics, pH(H_2O), electrical conductivity, and dissolved oxygen in the influent and the effluent were followed over time. The growth of the tomato plants and the quality (glucose, fructose, titratable acids, and Brix value) of the harvested tomatoes was also assessed. We demonstrated in a proof of concept experiment that a step-wise increase of the organic N-load resulted in comparable yields as a conventional system with a limited accumulation of free ammonia and ammonium levels potentially toxic for the plant. The results contribute to the practical application of organic fertilizers in soilless culture systems and its potential role towards a more sustainable horticulture.

1. Introduction

Nitrogen is a key element for any life form on earth (Butterbach-Bahl et al. 2011). Plants can use different simple inorganic nitrogen (N) forms such as ammonium (NH_4^+) and nitrate (NO_3^-) or more complex organic nitrogen forms such as amino acids and proteins (Näsholm et al. 2009). Ammonium is preferentially taken up by many plants when supplied in equimolar concentrations to nitrate (Gazzarrini et al. 1999) particularly when N-supply is low. An experiment with field grown tomatoes showed an inhibited root growth, when concentrations of ammonium were higher than 3.3 mg NH_4^+-N L^{-1} soil and 9.9 mg NO_3^--N L^{-1} soil (Bloom et al. 1993). Ammonium can be toxic and symptoms of ammonium toxicity range widely, and generally appear with external ammonium concentrations above 1.8 mg N L^{-1} to 9 mg N L^{-1} (Britto and Kronzucker 2002). The suppression of growth and yield in ammonium sensitive species like tomatoes can be severe, and for this reason, preventing ammonium toxicity is of major importance in soilless culture systems, when using organic fertilizers. Highest growth rates and plant yields are obtained by combined supply of both ammonium and nitrate (Marschner 2011).

Inorganic fertilizers have many known benefits in soilless culture systems (Sonneveld and Voogt 2009a). One of the keys to sustainable tomato growing is the supply of the correct amounts of nutrients at the correct time in relation to growth of a crop. Sonneveld and Voogt (2009b) showed that tomatoes have an uptake efficiency of 57% under free drainage conditions and up to 80% in closed systems. Commercially available organic fertilizers may offer extra benefits like an enhanced biological activity by favoring the root colonization by rhizosphere bacteria. These can improve N, P, K supply to the plant and mobilize low soluble nutrients (Hajdu et al. 2015) in comparison with inorganic fertilizers. Indeed organic nitrogen needs to be broken down to amino acids by proteases released by soil microorganisms (Miller and Cramer 2005). These different organic nitrogen forms can subsequently be transformed into ammonia (Wittebolle 2009) and further converted to nitrate. The delivery and production of readily available nutrients out of organic derived nitrogen due to microbial activity is difficult to predict and to control. Hence, this increases our need to investigate the potential of fertigation strategies to improve the nutrient flow in soilless culture systems in combination with organic fertilizers.

The electrical conductivity (EC) of the nutrient solution as well as the amount and frequency of fertigation (irrigation with nutrient solution) are the most important variables used by growers to control the supply of water and nutrients to the crop (Heinen et al. 2001). In combination with organic fertilizer the mineralization by microorganisms of the organic nitrogen is difficult to predict and consequently the electrical conductivity is a highly dynamic variable and difficult to control. At high EC, ample nutrients are available. Reduction of water uptake may occur due to osmotic effects in the irrigation water (highly negative osmotic potential), which may result in reduced crop growth. Tomatoes grown in combination with a constant supply of organic fertilizers show lower biomass production and yield and higher incidence of blossom end rot (BER) (Heeb et al. 2005a). Because of the mineralization of the organic nitrogen, ammonium can accumulate and cause ammonium toxicity. Consequently, control of the ammonium flux is a key element in adapted fertigation strategies (Thion et al. 2016). The aim of this proof of concept study was to analyze the nitrogen conversions from the growing medium and the response of the crop to an adapted fertigation strategy and an organic nitrogen source. We hypothesized, that in soilless tomato cultivation systems, a gradual increase of the organic nitrogen supply rate will result in comparable yields compared to inorganic fertilizers. In

this experiment, the nitrogen supply rate, the nitrogen source (organic and inorganic) were changed to explore the two following research questions for a tomato crop grown in an open soilless culture system in combination with mineral and organic growing media. (1) Does a step-wise increase of the inorganic nitrogen supply rate deliver enough nutrients to the plant resulting in comparable plant performance compared to conventional systems? (2) Does a step-wise increase of the organic nitrogen supply rate result in a comparable plant performance compared to a conventional system?

2. Material and Methods

2.1 Growing media and experimental setting

A plant experiment (8/9/2014-13/12/2014) was set up in the experimental glasshouse of Ghent University (50°59'36.6" latitude and 3°47'05.1" longitude). Two growing media were used: an organic (GB) and a mineral growing medium (RW). The organic growing medium (GB, Grow Bag, Peltracom, Belgium) was a mixture of sod peat [40% v/v], Irish peat [40% v/v] and coconut fiber [20% v/v]. Slabs of GB and mineral medium (RW, Rockwool, Grotop expert, Grodan, The Netherlands) had the following dimensions: 1.0 m × 0.2 m × 0.085 m and 1.0 m × 0.2 m × 0.075 m, respectively.

Solanum lycopersicum cultivar Admiro was grafted on *Solanum lycopersicum* L. x *Solanum habrochaites* Maxifort (Monsanto Vegetable Seeds, Bergschenhoek, The Netherlands). The young plants were raised in grow cubes made of blocking compost with the following dimensions: 0.1 m × 0.1 m × 0.08 m. The plant density was 5.1 plants m^{-2}. Based on preliminary experiments, the critical sample size for the determination of plant performance was determined (n=9 plants) with a power =82% and $P< 0.05$. samples were considered as independent samples as all the plants had their own drip irrigation system although three plants shared the same growing medium. We used five slabs for all the treatment GBOF Nvar, GBIF Nvar, RWIF Nar and RWIF Nmax and each slab contained three plants.

The growing media were placed in gutters with an inclination of 0°16'2". Day and night-time average temperature was 22.8±1.4°C and 20.2±0.55°C. The average daily CO_2-concentration was equal to 525.2±40.6 mg L^{-1}. The day and night average humidity was equal to 85.8% and 89.7±4.9%. Plants were treated against *Trialeurodes vaporariorum* with sticky traps and treated with 200 g L^{-1} myclobutanil and 103 g L^{-1} cyclohexanone (Systhane 20EW) against *Oidium*. Supplementary lighting (SON-T) of 100 µmol m^{-2} s^{-1} at plant level was given between 7h00 and 20h00, when the outdoor global irradiation was below 150 W m^{-2}.

2.2 Fertigation strategy

Both growing media (GB and RW) were subjected to a standard nutrient (de Kreij 1997) solution during the cultivation period based on an inorganic fertilizer (IF) with nitrate (92%) and ammonium (8%) as nitrogen source. The fertilizers were distributed according to a conventional A/B system with drip irrigation. In the organic fertilizer (OF) solution the inorganic nitrogen (ammonium and nitrate) was replaced for 100% by arginine (≥98%, Sigma Aldrich). The exact chemical composition of the two nutrient solutions for both experiments can be found in Table 5.1.

As shown in **Chapter 3**, the highest nitrate concentrations were found when the organic nitrogen concentration was below 315 mg N L^{-1} or 534 mg N $plant^{-1}$ d^{-1}. In addition, as shown by van der Ha (2013), a gradual increase of the ammonium concentration instead of the currently applied shock loads

has the highest potential to achieve a high ammonium tolerance of the microbial community. It was decided to gradually increase the nitrogen supply rate thereby not exceeding the maximum supply rate of 534 mg N plant^{-1} d^{-1}. It was decided not to test the mineral growing medium (RW) in combination with OF, because of the high ammonium concentrations of 652.9±52.1 mg NH_4^+-N L^{-1} causing ammonium toxicity at plant level and high free ammonia of 123 mg NH_3-N L^{-1} concentrations inhibiting the AOB (Anthonisen et al. 1976; Grunert et al. 2016b).

*Table 5.1: Composition of the organic (OF) and inorganic nutrient solution (OF) used for the growth of tomato plants. *pH of the organic (OF) and inorganic nutrient solution (IF) was correct to get the same pH of both nutrient solutions. **Nitrogen concentration was adapted according the nitrogen supply rate.*

Parameters	Organic nutrient solution (OF)	Inorganic nutrient solution (IF)
pH(H_2O)	6.0*	6.0*
Carbon content of arginine (mg C L^{-1})	36.2	0
C/N ratio of arginine	1.5	0
Conductivity (µS cm^{-1})	668	2080
Organic-nitrogen (mg N L^{-1})	240	0
Urea-nitrogen (mg N L^{-1})	0	0
Total Ammonia- nitrogen (mg N L^{-1})	0	19
Nitrate-nitrogen (mg N L^{-1})	0	221
Total-nitrogen (mg N L^{-1})*	**240**	**240**
Phosphorous (mg P L^{-1})	42	42
Potassium (mg K L^{-1})	376	376
Calcium (mg Ca L^{-1})	231	231
Magnesium (mg Mg L^{-1})	107	107
Sulphur (mg S L^{-1}l)	216	216

The organic (OF) and inorganic (IF) nitrogen supply rate was increased in 3 steps (Nvar): from 73 mg N plant^{-1} d^{-1} to 142 mg N plant^{-1} d^{-1}, from 142 mg N plant^{-1} d^{-1} to 218 mg N plant^{-1} d^{-1} and finally from 218 mg N plant^{-1} d^{-1} to 331 mg N plant^{-1} d^{-1}. This was tested in combination with the mineral (RW) and the organic growing medium (GB) resulting in the following treatments: GBOF Nvar, GBIF Nvar and RWIF Nvar. The macro and microelements were the same and not limiting for all the treatments. We used the mineral growing medium (RW) in combination with an inorganic fertilizer (IF) as a control (RWIF Nmax) with a nitrogen supply rate of 331 mg N plant^{-1} d^{-1} throughout the experimental period. This supply rate was based on a nitrogen concentration of 240 mg N L^{-1} according to the de Kreij (1997). The pH of the different fertigation solution was corrected to get the same pH for all the treatment. An overview of the fertigation strategy can be found in Figure 5.1.

Ammonium toxicity can be easily detected at plant level (visual control –see Picture 1 and 2). The ammonium concentration was tested on a regular basis and the free ammonia level was calculated. If the free ammonia concentration was within an acceptable range (Anthonisen et al. 1976), it was decided to increase the organic nitrogen supply rate. Anthonisen et al. (1976) reports inhibition of

nitrification at free ammonia concentrations of 10-150 mg N L^{-1}. Nitrobacter, responsible for the second part of nitrification can be inhibited at concentrations of 0.1-1.0 mg N L^{-1}. The plants were fertigated 6 times a day resulting in a water gift of 1.44±0.05 L $plant^{-1}$ d^{-1} and the effluent (n=3) from each slab was collected individually. The two outer slabs of each block were not selected, because of possible interactions with the adjacent rows. Because water is lost by evaporation and water is taken up by the plant, also the volume of effluent leaving the slabs was measured to calculate the nitrogen mass balance. We aimed at a drainage percentage of 30%, meaning that 30% of the water gift drained out of the slabs.

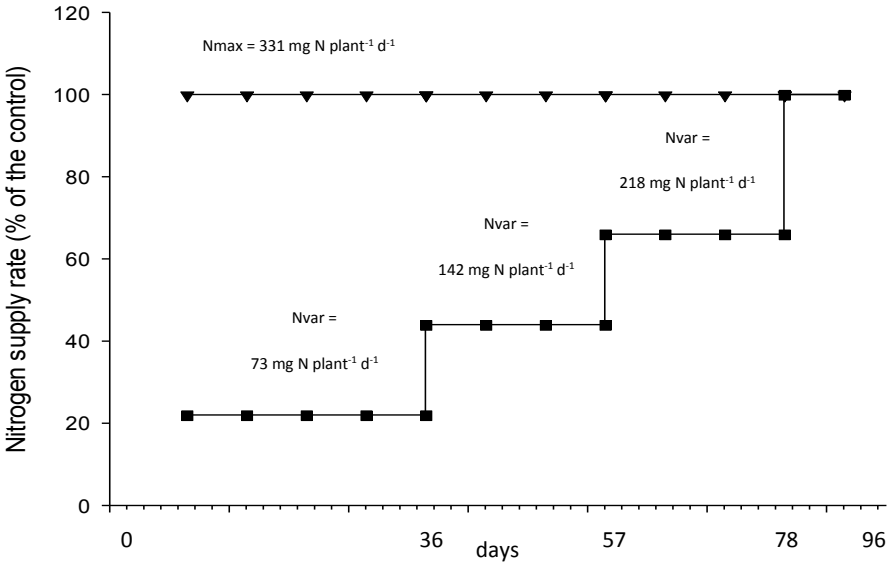

Figure 5.1: Overview of a three-step increase of the N-supply rate (22%-43% from day 0-36, 44%-66% from day 36-57, 66%-100% from day 57-78 and 100% from day 78-96) over a period of 96 days. Inorganic fertilizers were used as control at a maximum supply rate of 331 mg N $plant^{-1}$ d^{-1}.

2.3 Chemical analysis of the effluent

The chemical characteristics of the two growing media were determined at the start and the end of the experiments and performed as described by Gabriels et al. (1998b). Potassium, phosphorus, calcium, magnesium, iron and manganese were extracted (1:5 vol/vol) in ammonium acetate and measured with ICP. The electrical conductivity (EC), pH(H_2O), ammonium (NH_4^+), nitrate (NO_3^-), sulphate (SO_4^{2-}) and sodium (Na) were measured in a 1:5 v/v water extract according to EN 13038, EN 13037 and EN 13652, respectively. Nitrate was measured with an IC ion chromatograph. Ammonium was measured by steam distillation. The inner three out of the five slabs with the corresponding tomato plants were sampled to avoid confounding side effects. Drain water was collected after the first fertigation cycle and was immediately filtered through a 0.45 µm filter and stored in a falcon tube at 4°C until analysis. Effluent samples were taken once a week and nitrogen supply rate was increased after a sample of the effluent was taken. The concentrations of total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N) and total Kjeldahl-N (TAN + organic nitrogen) were determined as in Bremner and Keeney (1965). Nitrite and nitrate concentrations were determined in both influent and effluent using an ion

chromatograph (Metrohm, 930 compact IC flex, Herisau, Switzerland) and dissolved oxygen (DO) was measured with a portable meter (HQ40d, Hach Lange, Collorado, USA). Chemical oxygen demand (COD) was determined using the Photometer Nanocolor 500 D kit, following the manufacturer's instructions (Marcherey-Nagel).

2.4 Plant physiological measurements

The length of the plant (n=9) and actual stem diameter (n=9) were measured weekly with a measuring tape and a slide caliper (at the base of the stem, ±10 cm above the plant pot growing medium made of blocking compost and just above the place where the plants were grafted, respectively.

The first ripe tomatoes (n=15) of the vine were used for further analysis of sugar content and titratable acidity (Mencarelli and Saltveit 1988). Fruits were harvested, cut in four pieces and 2 quarters were immediately frozen at -80°C until further analysis for sugars and titratable acidity (Mencarelli and Saltveit 1988). The other pieces were used to determine the total dissolved dry matter or Brix value (°Bx) by a hand refractometer (Mencarelli and Saltveit 1988). About 200 mg fresh weight (FW) of tomatoes was used to extract the sugars with 80% ethanol at 70 °C for 10 min and 45°C for 3 h. After centrifugation at 5000g for 5 min, the supernatant was purified with 50 mg mL^{-1} polyvinylpolypyrrolidone (PVPP). The concentrations of glucose, fructose and sucrose were quantified by means of high performance anion-exchange chromatography with pulsed amperometric detection (KPAE-PAD, Waters) using a Carbo-Pac PA-100 column (Dionex) by using filtered (0.45 µm, Millipore) diluted samples. Titratable acidity was measured by titration with 0.1N sodium hydroxide and expressed as a citric acid percentage.

2.5 Statistical analysis

Growth parameters and sugar content were compared by one-way analysis of variance followed by Tukey's HSD test ($P< 0.05$). The normality of the distribution of this dataset was verified by checking the normal distribution of the error and the inspection of the constant variance. The homogeneity of variances was checked by Levene's test of equality of error variances. The normality of the distribution of this dataset was verified using Q-Q plot, a histogram, a box plot, checking for skewness and kurtosis. When the data were not normally distributed ($P<0.05$) and or there was no equal variance ($P<0.05$), we used the Kruskal-Wallis One Way Analysis of Variance on Ranks followed by an All Pairwise Multiple Comparison Dunn's test. All these analyses were conducted using SPSS version 22.0 (IBM Co., Armonk, NY, USA). Critical sample size of nine plants to detect differences in plant length was calculated based on a preliminary experiment (power =82% and $P<0.05$) taking into account that each plant was individually fertilized by drip irrigation but three plants shared a common slab comparable to commercial greenhouse settings. Differences in pH, electrical conductivity, dissolved oxygen, organic nitrogen, ammonium and nitrate supply and drainage rate among treatments were compared using a repeated measures mixed model in SAS (version 9.4, SAS Institute, Cary, USA) with the different tomato cultivating systems as a fixed effect and time. Hence, the differences in the pH, electrical conductivity, dissolved oxygen, nitrogen supply and drainage rates could be attributed time or tomato cultivating system or to the interaction of the two factors.

3. Results

3.1 Nitrogen dynamics, pH and EC in relation to the tomato fertigation strategy

We found a significant effect of treatment (RWIF Nmax, RWIF Nvar, GBIF Nvar, GBOF Nvar) (P<0.001) and time (P<0.001) and the interaction term (P<0.001) for the pH, electrical conductivity, dissolved oxygen, organic nitrogen, ammonium and nitrate supply and drainage rates (Table 5.2).

Table 5.2: pH, electrical conductivity (EC), dissolved oxygen (DO), nitrogen supply and drainage rates (mg N plant^{-1} d^{-1}) calculated for the effluent (supply) and the influent (drainage) in a three step increase of the N_{var}-load (22% - 44% - 66% and 100%) in function of time in combination with arginine as a simple organic nitrogen source. RW= mineral growing media; GB= organic growing media; IF= inorganic fertilizer; OF= organic fertilizer; N_{max}IF= variable inorganic nitrogen load in influent; N_{var}IF= variable inorganic nitrogen load in influent; N_{var}OF= variable organic nitrogen load of influent; N_{org}= organic nitrogen concentration in effluent; NH_4^+-N= ammonium concentration in effluent; NO_3^--N= nitrate concentration in effluent

	Parameters	Treatment (trt)				Effect		
		RWIF Nmax	RWIF Nvar	GBIF Nvar	GBOF Nvar	trt	Time	Treat* Time
INFLUENT (supply)	pH(H_2O)	6.01±0.02	6.00±0.02	5.97±0.02	6.13±0.02	<0.001	NS	<0.001
	EC (µS cm^{-1})	2022±25 ^{a}	972±25 ^{b}	977±24 ^{b}	293±23 ^{c}	<0.001	<0.001	<0.001
	DO (mg L^{-1})	7.2±0.03 ^{b}	7.5±0.03 ^{a}	7.4±0.03 ^{a}	4.9±0.03 ^{c}	<0.001	<0.001	<0.001
	Norg (mg N plant^{-1} d^{-1})	0±1 ^{a}	0±1 ^{a}	0±1 ^{a}	164±1 ^{b}	<0.001	<0.001	<0.001
	NH_4^+-N (mg N plant^{-1} d^{-1})	28±0.5 ^{a}	12±0.5 ^{b}	12±0.5 ^{b}	0±0.5 ^{c}	<0.001	<0.001	<0.001
	NO_3^--N (mg N plant^{-1} d^{-1})	318±4 ^{a}	148±4 ^{b}	155±4 ^{b}	0±4 ^{c}	<0.001	<0.001	<0.001
EFFLUENT (drainage)	pH(H_2O)	5.7±0.1 ^{d}	6.3±0.1 ^{c}	6.6±0.1 ^{b}	6.7±0.1 ^{a}	<0.001	<0.001	<0.001
	EC (µS cm^{-1})	2762±41 ^{a}	1181±42 ^{c}	1077±42 ^{c}	1926±42 ^{b}	<0.001	<0.001	<0.001
	DO (mg L^{-1})	6.4±0.2 ^{b}	6.1±0.2 ^{b}	6.7±0.2 ^{a}	5.4±0.2 ^{c}	<0.001	<0.001	<0.001
	Norg (mg N plant^{-1} d^{-1})	3.7±0.5 ^{b}	1.8±0.5 ^{c}	1.7±0.5 ^{c}	5.0±0.5 ^{a}	<0.001	<0.001	<0.001
	NH_4^+-N (mg N plant^{-1} d^{-1})	2.6±0.6 ^{b}	1.4±0.6 ^{c}	0.7±0.6 ^{c}	18.8±0.5 ^{a}	<0.001	<0.001	<0.001
	NO_3^--N (mg N plant^{-1} d^{-1})	245±15^a	89±15 ^{b}	62±15^c	6±15 ^{d}	<0.001	NS	NS
	Efficieny of N uptake (%)	27	42	61	81			

The differences in pH of the used fertigation solution were significantly different between the treatments, however smaller than 0.1 pH unit. The use of arginine seems to increase the pH of the organic fertigation solution OF. The pH of the drainage solution in general increased up to 0.5 unit with the only exception for RWIF Nmax, where the pH slightly decreased.

The electrical conductivity of the used fertigation solution differed significantly between the treatments. The use of arginine resulted in decreased values for the electrical conductivity with factor 3 compared to inorganic fertigation solution (980 µS cm^{-1}). The control treatment (RWIF Nvar) had the highest EC values of 2022±25 µS cm^{-1}. In general, the electrical conductivity of the drained IF and OF fertigation solution was higher compared to the supplied fertigation solution. The use of OF resulted in the highest EC difference between supplied and drained fertigation solution of more than 1600 µS cm^{-1}.

Dissolved oxygen (DO) values were on average 30-35% lower (4 mg O_2 L^{-1}) for OF compared to IF (6-8 mg O_2 L^{-1}) in the supplied fertigation solution, while the average DO values were on average 12-20% lower for OF compared to IF in the drained nutrient solution. The lowest DO values (4.9±0.8mg O_2 L^{-1}) were found with an N-supply rate of 142 mg N $plant^{-1}$ day^{-1} and were significantly different (P<0.001) from the control (6.7±0.4mg O_2 L^{-1}).

The control treatment with maximum N-supply rate (RWIF Nmax) showed that on average 8% of the supplied nitrogen was ammonium and 92% was nitrate, while the drained nitrogen consisted on average 1% ammonium and 99% nitrate. The same tendency was found for Nvar in combination with IF. GBOF, on the contrary, showed totally different nitrogen dynamics. The drained nutrient solution contained mainly ammonium (63%), nitrate (20%) and organic nitrogen (17%). The difference between supplied nitrogen and drained nitrogen is a first rough estimate of nitrogen recovered by the plants, thereby neglecting other potential losses, such as abiotic and biotic nitrogen immobilization, denitrification and volatization of ammonia. This means that plants potentially recovered 94.7 N $plant^{-1}$ d^{-1}, 67.2mg N $plant^{-1}$ d^{-1}, 101.9 mg N $plant^{-1}$ d^{-1} and 132.8 mg N $plant^{-1}$ d^{-1}, for RWIF Nmax, RWIF Nvar, GBIF Nvar and GBOF Nvar respectively.

3.2 Plant performance

No significant differences between the treatments were found for the specific leaf area (SLA) and the dry matter content of the leaves. The plant length was significantly (P<0.05) different between GBIF and the two other treatments GBOF and RWIF after a period of 23 days. The plant length of RWIF and GBOF was not significantly different. The average final plant length in the three-step increase (Figure 5.2) of the N-supply rate was 261±34 cm for RWIF with Nmax, 293±22 cm for GBOF with Nvar, 258±18 cm for RWIF with Nvar and 272±25 cm for GBIF with Nvar.

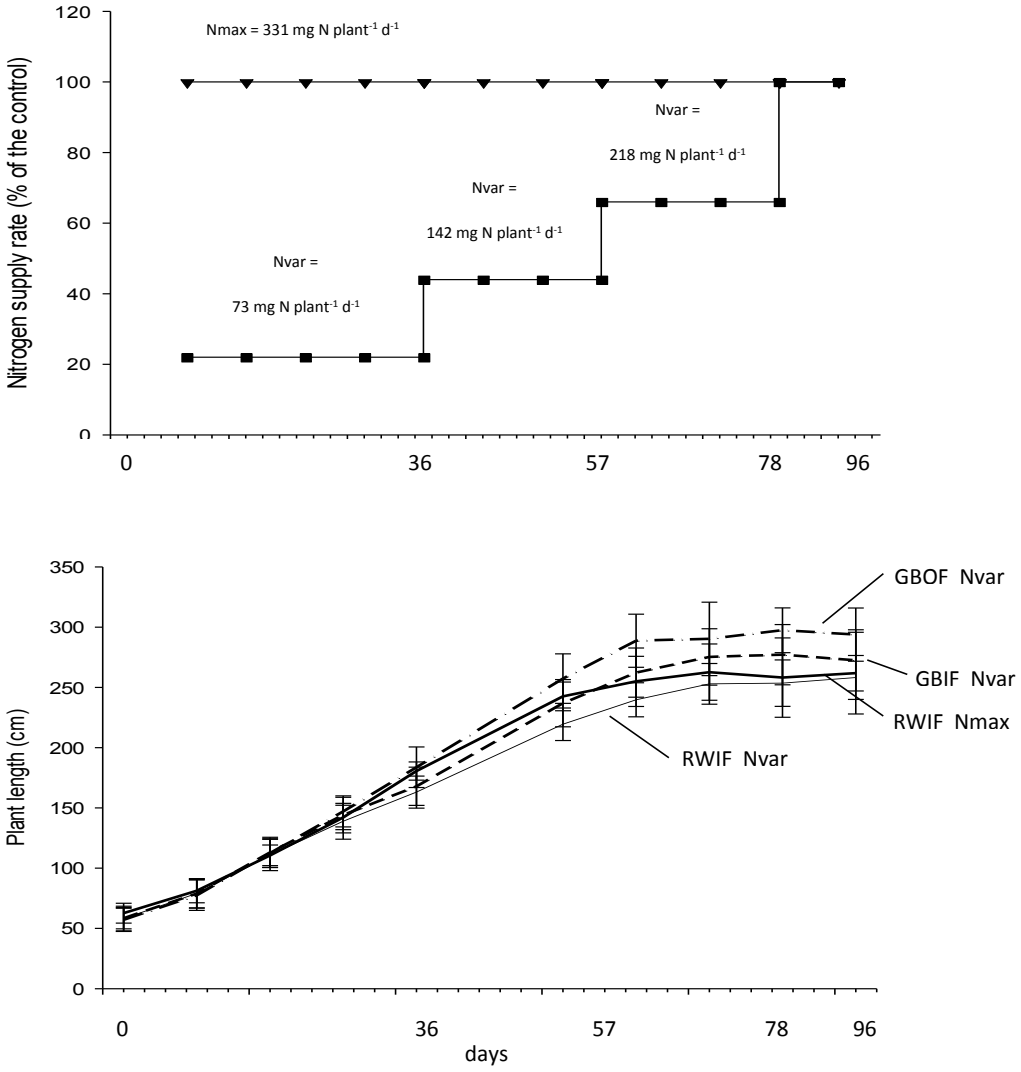

Figure 5.2: Evolution of the plant length of a tomato plant in a three-step increase of the N_{var}-load in function of time in combination with arginine as a simple organic fertilizer; RW= mineral growing media; GB= organic growing media; IF= inorganic fertilizer; OF= organic fertilizer

Significant differences between the treatments were found after 30 days (P<0.05). The average stem diameter in the three-step increase of the N-load was 11.6±1.3 mm for RWIF with Nmax, 10.8±1.4 mm for GBOF with Nvar, 11.1±1.5 mm for RWIF with Nvar and 11.7±1.0 mm for GBIF with Nvar. No significant differences were found between treatments.

Picture 1: Pictures of leaves, stems and root system of plants fertilized with IF

Picture 2: Pictures of leaves, stems with lesions and root system of plants fertilized with OF (red oval)

Necrotic lesions of the stem and the leaf petioles and the laminae result from an accumulation of ammonia in combination with pH(H_2O) values lower than. Plants fertilized with OF (marked with red oval) have a weaker rooting system, exhibited a stunted growth, develop and curled leaves and stem lesions (Picture 2) compared to plants fertilized with IF (Picture 1). These findings were also confirmed

in preliminary plants test in 2013 and 2014. The tomato plants subjected to a three-step increase in N-supply rate did not develop severe necrotic stem lesions even after a prolonged nutrition with arginine.

3.3 Tomato quality

The average fresh fruit weight in a three-step increase of the N-load was significantly higher with RWIF Nmax and GBIF Nvar in comparison with RWIF Nvar and GBOF Nvar, which had the lowest fresh tomato weight. GBIF Nvar had a significant higher amount of green tomatoes (P<0.05), red tomatoes and total yield (P<0.01) than GBOF Nvar, RWIF Nvar and RWIF Nmax (Table 5.3). GBOF Nvar had a similar total yield like RWIF Nmax; however yield of green tomatoes was significantly higher. Tomato fruits grown with IF had the highest significant TSS (°Bx) compared to OF (P<0.05). Furthermore, the titratable acidity was significantly higher in RWIF with Nmax. Interestingly, glucose and fructose concentrations of GBOF were comparable with the control treatment RWIF Nmax. The glucose/fructose ratio should be about 1 (Yilmaz 2001) and the GBIF treatment had the highest ratio of 0.88 and RWIF had the lowest ratio of 0.8 (Table 5.3).

Table 5.3: Aerial biomass and quality of tomatoes in function of different fertigation strategies (N_{max} and N_{var}), two different growing media (mineral and organic) and two different nitrogen forms in a three-step fertigation system. Different letters above numbers indicate significant differences at P = 0.05 (Tukey LSD test). (n =15). RW= mineral growing media; GB= organic growing media; IF= inorganic fertilizer; OF= organic fertilizer; N_{max}= maximum nitrogen load; N_{var}= variable nitrogen load

	RWIF N_{max}	**RWIF N_{var}**	**GBIF N_{var}**	**GBOF N_{var}**
Fresh weight red tomatoes (g plant^{-1})	132.9±51.4^{a}	110.0±34.8^{b}	135.±43.16^{a}	88.8±37.5^{c}
Total yield of red tomatoes (kg m^{-2})	2.76±1.56^{a}	2.11±0.99^{a}	3.05±1.70^{a}	2.72±1.19^{a}
Total yield of green tomatoes (kg m^{-2})	1.23±1.02^{a}	1.71±1.69^{a}	2.59±1.23^{b}	1.82±0.69^{ab}
Total yield of tomatoes (kg m^{-2})	4.27±1.53^{ab}	3.30±1.10^{a}	5.64±1.75^{b}	4.41±1.52^{ab}
Tomatoes with Blossom End Rot (%)	3	2	0	2
Total Soluble Solids (°Bx)	5.1±0.55^{a}	4.37±0.39^{c}	4.5±0.45^{b}	4.16±0.65^{d}
Titratable Acidity (meq 100 mL^{-1})	0.11±0.03^{a}	0.1±0.02^{b}	0.1±0.02^{b}	0.09±0.025^{b}
Glucose (g 100 g^{-1} fresh weight)	0.23±0.14^{ab}	0.16±0.12^{b}	0.19±0.15^{a}	0.19±0.15^{ab}
Fructose (g 100 g^{-1} fresh weight)	0.27±0.17^{a}	0.20±0.15^{a}	0.32±0.21^{a}	0.23±0.18^{a}
Ratio (dry matter acid^{-1})	46.4	43.7	4 5.0	46.2
Ratio (glucose fructose^{-1})	0.85	0.80	0.88	0.83

4. Discussion

Our major objective was to develop adapted fertigation strategies in soilless culture systems in combination with organic fertilizers and organic growing media. We hypothesized, that in soilless

tomato cultivation systems, a gradual increase of the organic nitrogen supply rate will result in comparable yields compared to inorganic fertilizers. In this experiment, the nitrogen supply rate, the nitrogen source (organic and inorganic) were changed to explore the two following research questions for a tomato crop grown in an open soilless culture system in combination with mineral and organic growing media.

4.1 Does a step-wise increase of the inorganic nitrogen supply rate deliver enough nutrients to the plant resulting in comparable plant performance compared to conventional systems?

The use of an adapted fertigation strategy of RWIF Nvar in comparison to RWIF Nmax resulted in a significant lower plant length (P<0.05), lower yield (P<0.05), fresh weight of the tomatoes (P<0.05), total soluble solids (P<0.05), titratable acidity (P<0.05), and glucose concentration (P<0.05) in the tomatoes. We found significant lower values for the electrical conductivity (P<0.001), dissolved oxygen (P<0.001), ammonium (P<0.001) and nitrogen supply rate (P<0.001) for RWIF Nvar. We also found significant differences in the nitrogen drainage rates between RWIF Nmax and RWIF Nvar. Based on the results of the nitrogen supply and nitrogen drainage rates, we may assume higher nitrogen uptake rates by the plants in combination with RWIF Nmax (94.7 mg N $plant^{-1}$ d^{-1}) than with RWIF Nvar (67.8 mg N $plant^{-1}$ d^{-1}). The results clearly indicate that we have to reject the first hypothesis, indicating that a step-wise increase of the nitrogen supply rate in combination with a mineral growing medium and an inorganic fertilizer solution is not a good option, as they result in lower yields and worse plant performance. The step-wise increase of the nitrogen supply rate resulted in higher potential nitrogen uptake rates of the plant. These differences can be explained by the fact that we have a linear relationship between the yield of crops and the uptake of nutrients by tomato plants (Sonneveld and Voogt 2009a). Crops grown in soilless culture systems are generally grown at high external nutrient concentrations. High nutrient concentrations in the external solution does not significantly affect the uptake (Sonneveld and Welles 2004). The electrical conductivity (EC) of the nutrient solution as well as the amount and frequency of fertigation (irrigation with nutrient solution) are the most important variables used by growers to control the supply of water and nutrients to the crop. The amount and the frequency were not the same for both RWIF Nvar and RWIF Nmax. As shown by Heinen et al. (2001) at lower EC values (≤2000 µS cm^{-1}) growth is reduced due to limitations in nutrient availability (transport rate towards the root) while at too high EC values (≥ 4000 µS cm^{-1}) growth is reduced mainly due to water stress (low osmotic potential).

Interestingly, the use of an organic growing medium in combination with an inorganic fertilizer solution did not show the same trend as the mineral growing medium. On the contrary, we found increased yields (P<0.05) and plant length (P<0.05). GBIF Nvar scored significantly lower for the total soluble solids (P<0.05), titratable acidity (*P<0.05*), and glucose concentration (*P<0.05*) in the tomatoes. The incidence for blossom end rot was also lower in combination with GBIF Nvar than with RWIF Nmax, which is in agreement with Grunert et al. (2008). We observed more roots (data not shown) in combination with GB than with RW, indicating a higher root surface than can be used by the plant to take up nutrients and nitrogen. The small root volumes used in soilless culture systems are responsible for tremendous fluctuations in the salt and nutrient status of the root environment(Sonneveld and Voogt 2009a). Another explanation for the differences between GB and RW might be explained by the fact that the hydraulic and physical properties of RW and GB are different with respect to water availability for the plant. The volumetric water content of RW is very high near saturation, but

decreases significantly with decreasing matric potential. Because of this low water buffering capacity, hydraulic conductivity decreases dramatically with decreasing h. Such a decrease in hydraulic conductivity might cause water and nutrients to be less available for plant roots, even at slightly negative h-values and finally jeopardizing the yield (De Swaef et al. 2012). In this study, it was clear that a gradual increase of the inorganic nitrogen supply rate resulted in a decreased plant performance and that this could be attributed to the lower supply of nutrients.

4.2 Does a step-wise increase of the organic nitrogen supply rate result in a comparable plant performance compared to a conventional system?

The use of an adapted fertigation strategy of GBOF RWIF Nvar in comparison to RWIF Nmax resulted in a significant higher plant length (P<0.05) and comparable yield. We found a significant lower fresh weight of the tomatoes (P<0.05), total soluble solids (P<0.05), titratable acidity (P<0.05), and glucose concentration (P<0.05) in the tomatoes. We found significant lower values for the electrical conductivity (P<0.001), dissolved oxygen (P<0.001), organic nitrogen (P<0.001) supply rate for GBOF Nvar in comparison to RWIF Nmax. We also found significantly higher amount of organic nitrogen (P<0.05), ammonium (P<0.05) and pH values (P<0.05) in GBOF Nvar in comparison to RWIF Nmax. Electrical conductivity (P<0.05), dissolved oxygen (P<0.05) and nitrate (P<0.05), on the contrary, was significantly lower in the drained fertigation solution. Based on the results of the nitrogen supply and drainage rates, we may assume a nitrogen uptake rate by the plants in combination with GBOF Nvar of 134.2 mg N $plant^{-1}$ d^{-1}, which is 1.4 higher than with RWIF Nmax. Based on these results we can accepted the second hypothesis with respect to yield and plant length and reject it with respect to the quality of the tomatoes.

Control of the ammonium flux is a key element in adapted fertigation strategies (Thion et al. 2016) in combination with organic fertilizers. Solid and liquid organic fertilizers can be considered as slow release fertilizers and they rely on microbial activity for mineralization (Hajdu et al. 2015). The organic N present in organic fertilizers is first mineralized to ammonium (NH_4^+) (Jansson 1958) and plants are sensitive to ammonium. The threshold, at which symptoms of toxicity become visible, differs widely among plant species. Plants most sensitive to ammonium (NH_4^+) toxicity are tomatoes (Britto and Kronzucker 2002). A three-step increase in N-supply rate with arginine resulted in a maximum free ammonia concentration of 1.5 mg NH_3-N L^{-1} and a maximum ammonium concentration of about 100 mg NH_4^+-N L^{-1}. Decreased ammonium concentrations in the drain can be explained by the fact that part of the ammonium is immobilized by the GMC. Significant dissociation of ammonium takes place at pH values higher than 6.25. At this value, approximately 1‰ of the ammonium is dissociated to ammonia, potentially resulting in ammonia volatization. Free ammonia has not only an effect on the ammonia and the nitrite oxidizing bacteria, but can impact plant growth (Britto and Kronzucker 2002). Mattsson and Schjoerring (1996) demonstrated that ammonium-grown plants had increased ammonium levels in both shoots and roots and also increased ammonium concentrations in xylem sap. These high concentrations can result in necrotic lesions and a stunted growth of the tomato plants. In addition, ammonium toxicity can be alleviated by co-provision of nitrate (Britto and Kronzucker 2002) and our results indicate nitrification activity. We found a low amount of necrotic lesions. This decreased number of necrotic lesions and the superior plant length might be explained by the adapted fertigation strategy leading to a decreased accumulation of ammonium and indicating that the increased ammonium concentrations might have been alleviated through the formation of nitrate. These results are in contrast to Heeb et al. (2005a), where the organic treatment showed lower biomass production

with fixed N-supply rates ranging from 35 to 142 mg N plant^{-1} d^{-1}. The use of the organic fertilizer (OF) did not result in increased glucose and fructose concentrations. This is in contrast with Siddiqi et al. (2002), who observed improved taste of tomatoes when fertilizers contained ammonium, and suggested that this improvement could be due to elevated levels of glutamine and glutamate in the tomato tissue (Heeb 2005; Heeb et al. 2005b). Taste is a complex phenomenon and depends on the composition of sugars and acids. The composition of the sugars and acids can be influenced by the fertigation and nitrogen form provided, the cultivar, the light and temperature conditions, because of their effect on biosynthesis and metabolism within the fruits (De Swaef 2011; Ho 1996; Hobson and Davies 1976). As shown by Heinen et al. (2001), EC values of about 2000 µS cm^{-1} are very likely to result in reduced growth due to limitations in nutrient availability or due to water stress. GBOF on the contrary, showed increased EC values, indicating that arginine is mineralized as a consequence of microbial activity, i.e. ammonification of the organic nitrogen in GBOF. This is also supported by the increased pH values of GBOF. Due to mineralization process, ammonia is released and depending to the pH of the growing medium directly withdraws an H^+ to form ammonium (NH_4^+) and this causes an increase of the pH in the growing medium.

Indeed this adapted fertigation strategy in combination with organic fertilizers is needed not only with respect to plant growth, but also due to the fact that the mineralization of the organic nitrogen is a biological driven process. The gradual increase of the ammonium concentration instead of the currently applied shock loads have high potential to achieve a high ammonium tolerance and are in agreement with the results of van der Ha (2013). Due to the scope of the work, the applied adapted fertigation strategy seems to be a promising approach to narrow the yield gap between a soilless culture system in combination with inorganic and organic fertilizers and represents the basis for prolonged field trials with tomatoes. These results support our hypothesis that with a step-wise increase of the organic nitrogen supply rate plant performance is positively affected and ammonium toxicity is limited compared to a conventional system with inorganic fertilizers. By doing so, the plants become accustomed to increased ammonium concentrations, the mineralization of organic N is enhanced and the ammonium flux is increased by the soilless community. The results indicate that the proper selection of the organic fertilizer is needed and in addition, mineralization experiments are needed to predict the mineralization rate of the specific organic fertilizer in the organic growing medium. In addition, the use of organic fertilizers offers advantages within a broader sustainability framework. There is a benefit for the environment due to an enhanced recycling of organic materials in commercially available granular and liquid organic fertilizers and a reduced need of mineral fertilizers with a high carbon footprint (3.6 kg CO_2-eq kg^{-1} N produced) (Yara 2012). Additional large-scale plant trials in combination with true organic fertilizers instead of arginine are needed to proof their potential application in soilless culture systems.

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CHAPTER 6: TEMPORAL VARIABILITY OF MICROBIAL COMMUNITIES BETWEEN FOUR CONTRASTING SOIL AND SOILLESS TOMATO CULTIVATING SYSTEMS

Oliver Grunert, Emma Hernandez-Sanabria, Nele Ameloot, Tom Beyers, Stefaan De Neve, Marie-Christine Van Labeke, Dirk Reheul, and Nico Boon. Temporal variability of microbial communities between four contrasting soil and soilless tomato cultivating systems.
(*In preparation for submission*)

Abstract

Soil and soilless culture systems are highly dynamic environments. Thus, the microorganisms are associated with these systems face changing soil and soilless culture conditions. These changing conditions, including nitrogen fertilization, often having effects on the structure of soil microbial communities (Ramirez et al. 2010). In this work, we examined how the diversity and composition of bacterial communities changed across a 10-month period in four different soil and soilless tomato cultivating systems. We used PLFA and high throughput sequencing of the 16S rRNA gene to compare the diversity and community composition within and between cultivating systems over time. Given that soil fertilization and soilless fertigation influence bacterial communities, we hypothesized that each tomato cultivating system would harbor a distinctly different bacterial community at the start and changes over time in total species, evenness and diversity is different between four contrasting tomato cultivating systems as a result of different fertilization strategies. In this study, community structure was distinctly and consistently different between the organic soil and the soilless culture system. These differences could be attributed to differences in chemical characteristics of the four tomato cultivating systems. The variability over time needs to be carefully assessed when comparing microbial diversity between tomato cultivating systems and fertilizer application may be responsible for variations overtime in the ecosystem observed. Molecular and chemical analysis can provide insight into the factors influencing the overall diversity of soil and soilless microbial communities.

1. Introduction

Soilless culture systems play a central role in horticulture. Soilless culture systems have proven its merits in terms of reliability, high plant productivity, reduction of soil-borne pathogens and the control over water and nutrient supplies. However, it suffers from drawbacks such as the high costs and skills, the high demand for and use of inorganic nutrients, the use of pesticides and the risk of spreading root diseases through the system. In contrast, organic cropping systems depend on natural mechanisms for plant nutrition and crop protection. Management practices are primarily directed at sustaining or improving soil fertility, with compost additions and animal and green manures (Willekens 2016). The major part of soil nitrogen is in organic forms and is converted by microorganisms into a plant available form. Organic cropping systems suffer from major drawbacks such as lower yields compared with conventional cropping systems (Reganold and Wachter 2016), presence of soil borne diseases and low control over water and nutrient supplies. However, these systems deliver nutritious foods that contain less (or no) pesticide residues, compared with conventional cropping systems (Reganold and Wachter 2016). Organic agriculture has an important role when it comes to the establishment of sustainable farming systems, but no single approach, neither soilless culture systems nor organic cropping systems will safely feed the planet. Rather, a blend of organic and other innovative cultivating systems is needed (Reganold and Wachter 2016).

Soil and soilless culture systems are highly dynamic environments where the microorganisms associated with these habitats face changing conditions. It is reported that soils under cultivation have different communities from those found in non-cultivated systems (Jangid et al. 2008; Wu et al. 2008). These changing conditions, including inorganic and organic nitrogen fertilization, have effects on the structure of soil microbial communities (Ramirez et al. 2010). In organic soils, fertilizers, such as compost and manure, are often applied as a one-time base dressing as well for top dressings. In soilless culture systems in combination with growing media, more or less all the fertilizers are applied by fertigation on a daily basis and depending on solar irradiation. There is a limited understanding of variability of the soil and soilless microbial community composition over time and differences between different cultivating systems. In soil systems, research focused on temporal variability in microbial driven nitrogen conversion processes across time (Lauber et al. 2013), however this kind of research is scarce in soilless culture systems. The effects of organic farming on microbial diversity are uncertain, in particular because the experimental systems and management definitions vary widely (Hartmann et al. 2015). Some studies observed an increase of richness and a decrease in evenness (Hartmann et al. 2015), while other studies reported an increase in richness and an increase or no effect on evenness after manure amendment (Hartmann et al. 2015). High-throughput sequencing studies reported an increase in microbial evenness in organic farming systems (Sugiyama et al. 2010), but have not detected significant effects on richness (Sugiyama et al. 2010).

Composition of microbial communities can influence the rates of nitrogen conversion and their response to changing environmental conditions (Schulze et al. 2001). Thus, improved understanding of the variability over time in soil and soilless microbial communities will assist in understanding their functions and changes between different cultivating systems. Generally, analyses of soil and soilless microbial communities can provide insight into the factors influencing the overall diversity. While gradual progress is made to understand the microbial ecology of (organic) soils, a large knowledge gap exists concerning soilless microbial communities and their structure. In this work, we examined how the diversity and composition of soil and soilless bacterial communities changed across a 10-month

period from plots representing four contrasting tomato cultivating systems. We used PLFA and high throughput sequencing of the 16S rRNA gene to compare the diversity (total species, evenness and diversity) and community composition within and between these four different tomato cultivating systems over time. Given that soil and soilless culture systems in combination with organic growing media harbor distinctly different bacterial communities and given that nitrogen fertilization and fertigation, effects the microbial communities composition, we hypothesized that each tomato cultivating system would harbor a distinctly different bacterial community at the start and changes over time in total species, evenness and diversity is different between four contrasting tomato cultivating systems as a result of different fertilization strategies. The first soilless culture system was the "aquaponics" system (GBFISH), the second soilless culture system was an organic growing medium in combination with organic fertilizers (GBOF), the third system was an organic soil supplemented with animal manure, i.e. blood meal (SOILANIMAL) and the forth system was an organic soil supplemented with plant manure, i.e. malt sprouts (SOILPLANT).

2. Materials and Methods

2.1 Experimental setup for the organic soil and soilless culture system

The organic growing medium (GB, Grow Bag, Peltracom, Belgium) was a mixture of white peat (H2-H4 on the von Post scale (Von Post, 1926) [40% v/v sod peat and 40% v/v Irish peat]) and coconut fiber ([20% v/v]). Slabs of GB had the following dimensions: 1.0 m × 0.2 m × 0.085 m and one slab contained 5 tomato plants with an organic blocking compost (10cm x 10cm x 6cm) as plant pot growing medium for each plant. An organic soil (PCG Kruishoutem) was used for the other two tomato cultivating systems (Supplementary table 6.1). The soil at the experimental site is a Haplic Podzol (Baxter 2007) with a loamy sand texture (85% sand, 11% silt and 4% clay) (Arthur et al. 2011).

The tomato *Solanum lycopersicum* cultivar RZ 72-704 (Rijk Zwaan, Fijnaart, The Netherlands) was grafted on *Solanum lycopersicum* L. x *Solanum habrochaites* Maxifort (Monsanto Vegetable Seeds, Bergschenhoek, The Netherlands). Tomatoes were sown on 18/12/2014, planted on 11/2/2015 and the first harvest started on the 5/5/2015 and ended on the 4/11/2015. Plant density was of 2.65 plants m^{-2} (*i.e.* 1 plant per 0.47m x 0.8m) was equal among treatments. Each row contained 6 slabs and only the inner 4 slabs were used for sampling to avoid confounding effects.

Four contrasting cultivation systems were compared with each other (GBOF, GBFISH, SOILANIMAL and SOILPLANT). The soilless cultivation system was used in combination with an organic growing medium (GB) (Supplementary figure 6.1 – S91) and two different fertilizer solutions were used, *i.e.* organic fertilizer (GBOF) and fish effluent (GBFISH). For the organic soil also two different kind of fertilizer were used. One part of the organic soil was fertilizer with plant-derived (malt sprouts) material (SOILPLANT) (Supplementary figure 6.1 – S93) and the other part with animal-derived (blood meal)) material (SOILANIMAL) (Supplementary figure 6.1 – S92) at the start of the experiment. Forty plants were used per treatment (GBOF, GBFISH, SOILPLANT and SOILANIMAL), which was equal to an effective experimental surface of 15.1 m^2 per treatment. Water gift was followed for all the tomato cultivating systems (Supplementary figure 6.5).

The plant experiment was set up in the experimental glasshouse of the Provinciaal Proefcentrum Groenteteelt in Kruishoutem (longitude = 3°31'E and latitude = 50°56'N and 10 meter above sea level). The glasshouse was divided in three compartments (S91-S92 and S93) and every compartment had a surface of 80 m² with the same climatic conditions. An overview of the experimental set-up can be found in Supplementary figure 6.1. The glasshouse experiment started the 11th of February 2015 and ended on November the 4th 2015. The plants cultivated in the organic soil had about 21 L soil plant $^{-1}$ at their disposal, assuming that the plants used between 0-8.5 cm of the top layer for rooting. This contrasts to the soilless culture system, where the plants had about 3.4 L growing medium plant^{-1} at their disposal.

2.2 Fertigation of the four different tomato cultivating systems

Fertigation of tomatoes is based on solar irradiation considering the drained water. Irrigation varies between 3 mL J^{-1} and 4 mL J^{-1} at higher temperatures resulting in a water gift of between 8 and 10 L m^{-2} d^{-1}. The fertigation for GBOF was without recirculation and it was estimated at the start of the experiment that about 1300 L m^{-2} water was needed for the cultivation of tomatoes during a whole season (February until November). The potential for using the nutrient-rich process water coming from the fish farming as a nutrient solution for the cultivation of tomatoes is mainly dependent on the chemical composition of the nutrient solution. Ammonia is the main excretion product of the fish. The excreted ammonium is converted into nitrate and is used as the primary inorganic nitrogen source for the tomato plants. The fertigation solution coming from FISH was amended and corrected when needed aiming at a final composition of 0.7 mmol NH_4^+ L^{-1}, 18.4 mmol NO_3^- L^{-1}, 10.9 mmol K L^{-1}, and 6.2 mmol Ca L^{-1}, 2.8 mmol Mg L^{-1}, 0.7 mmol Cl L^{-1}, 5.1 mmol SO_4^{2-} L^{-1} and 1.7 mmol $H_2PO_4^-$ L^{-1}. This fertigation solution was used for GBFISH.

For the fertigation of GBOF, four different organic fertilizers (ANTYS MgS, Biosyr, Nutrikali, and SP; Frayssinet, France) were combined with each other aiming at a balanced nutrient solution suitable for the cultivation of tomatoes. Moreover, a N:P:K ratio of 1:0.2:1.6 was respected throughout the whole experimental period for GBOF and GBFISH. Nutrient solution for GBOF was supplemented with extra calcium chloride ($CaCl_2$) and Libremix (3,2% Fe-EDTA, 1,5% Mn-EDTA, 1,6% Cu-EDTA, 0,6% Zn-EDTA, 0,8% B and 2,5% Mo; Brinkman, The Netherlands) if needed, such as increased incidence of blossom end rot (BER). The nitrogen concentration of the nutrient solution was increased or decreased according to the growth of the plants and/or the presence or absence of deficiency symptoms, such as blossom end rot (BER).

SOILANIMAL was supplemented with 252 kg N ha^{-1} coming from blood meal with a total nitrogen content of 8% and 1630 kg ha^{-1} of patentkali (30% K_2O, 10% MgO and 42% SO_3), while SOILPLANT was supplemented with 300 kg N ha^{-1} coming from malt sprouts and 1630 kg ha^{-1} of patentkali (30% K_2O, 10% MgO and 42% SO_3) at the start of the experiment. Chemical composition of the organic soil (SOILPLANT and SOILANIMAL) and the soilless culture system in combination with the organic growing medium can be found in Supplementary table 6.1 and Supplementary table 4.1. Detailed information about the type, the amount and composition of fertilizer used can be found in Supplementary table 6.2, Supplementary table 6.3 and Supplementary table 6.4.

Although the irrigation strategy was the same for the soilless culture systems, drain water was reused in GBFISH but not in GBOF resulting in an increased water use in GBOF. Nitrogen supply rate per square meter increased steadily from 1.3 g N m^{-2} d^{-1} to 112.6 g N m^{-2} d^{-1} between days 78 and 161 for GBOF.

Nitrogen load was decreased to 20 g N m^{-2} d^{-1} in the following next 27 days as a result of increased blossom end rot (BER) incidence, development of smaller leaves, reduced plant growth, and leaf chlorosis. Nitrogen supply rate was increased again up to 164 g N m^{-2} d^{-1} after the above mentioned period. In contrast, irrigation strategy in the soil was based on the water content, which was visually checked by a soil drill according to the experience of the people of the experimental station. Overall, the different tomato cultivating systems were treated and fertilized independently from each other. The only common factors used were the same plants, the same climatic conditions, the same planting date and plant density.

2.3 Nitrogen determination in the soil, soilless culture system and plant

The physicochemical characteristics of the different growing media were determined at the start and during the growing season. The chemical analysis was performed as described by Gabriels et al. (1998b). Potassium, phosphorus, calcium, magnesium, iron and manganese were extracted (1:5 vol/vol) in ammonium acetate and measured with ICP. The electrical conductivity (EC), pH(H_2O), ammonium (NH_4^+), nitrate (NO_3^-), sulphate (SO_4^{2-}) and sodium (Na^+) were measured in a 1:5 v/v water extract according to EN 13038, EN 13037 and EN 13652, respectively. Nitrate was measured with an IC ion chromatograph. Ammonium was measured by steam distillation. Only the inner four slabs out of six slabs with the corresponding tomato plants were sampled, to avoid confounding effects. Supplementary analyses on the soil were executed to determine plant available nutrients (NH_4^+, NO_3^-, the dry matter content) in the 0-10 cm profile by shaking 5 g air-dried soil in 100 ml ammonium lactate for 4 hours (Egnér et al. 1960). The elements were measured using a CCD simultaneous ICP-OES (VISTA-PRO, Varian, Palo Alto, CA). Whole plants supplemented with the fertilizers (GBOF and GBFISH) were harvested at 27/7/2015 and 4/11/2015, chopped and samples from stem and leaves without tomatoes were collected. The total nitrogen content of the plants sampled was determined according to Dumas (13654-2).

2.4 Estimation of the nitrogen dynamics in soil and soilless culture systems

The ammonium and the nitrogen content in the 0-10 cm organic soil layer (Nmin) were calculated based on the ammonium and the nitrate concentration at 11/2/2015 (time point 1), 27/7/2015 (time point 2) and 4/11/2015 (time point3) and the estimated soil dry bulk density (1.25 kg L^{-1}) of the 0-10 cm soil layer. As shown by Zotarelli et al. (2009) in an experiment with tomatoes at the beginning of the reproductive phase about 70–75% of the total root length density was concentrated in the 0– 15 cm soil layer, while 15–20% of the roots were found in the 15–30 cm layer. Corresponding root length distribution values during the reproductive phase were 68% and 22%, respectively. Root distribution in the soil profile thus appears to be mainly driven by development stage, soil moisture and nutrient availability. Fertilizers were applied in the top layer of the organic soil (0-10 cm) and soil water content was controlled in the 0-10 cm layer. In addition, we determined in the organic soil at a depth of 10 cm a root impenetrable soil layer and no roots were detected below 0-10 cm. The ammonium and the nitrate concentration of GBOF and GBFISH were also determined and were recalculated based on the amount of growing medium needed per ha, i.e. 90 m^3 ha^{-1}.

The N uptake by the tomato plants was measured at time point 2 and time point 3. At time point 2 and 3 the dry matter and N content of whole plant samples were determined for calculation of dry biomass and total N uptake at time point 2 and 3. Samples were taken from shredded tomato plants (n=4).

Samples were oven dried in a ventilated oven at 70°C during at least 48h. N content was determined on ground dried plant material using the Kjeldahl method (ISO 5983-2).

The balances of plant available nitrogen, i.e. ammonium and nitrate, were calculated by subtracting the N supply by fertigation from the mineral N, i.e. ammonium and nitrate, taken up by the plants, resulting in the "apparent" net N mineralization (ANM) according to Feller and Fink (2000). However, plants are known for their capacity to take up organic nitrogen (Näsholm et al. 2009). Three periods were used for the calculation of the balance (11/2/2015 - time point 1, 27/7/2015 - time point 2 and 4/11/2015 - time point3). The N input through planting material was the same for all the objects and was negligible. In the assumption that no or minor N losses occur between the considered sampling occasions, ANM is the net N release from both soil organic matter in the organic soil and the organic growing medium and organic matter applied by fertilization (Willekens 2016) in SOILANIMAL and SOILPLANT and the nitrogen fertilization for GBOF and GBFISH. With respect to the cultivation period of the tomatoes, an estimated balance of plant available N was calculated by subtracting nitrogen supply from nitrogen taken up by the plant and was expressed in kg ha^{-1} (Table 6.1). Based on the research by (Beck et al. 1995; Scharf and Weier (1994)) it was shown that tomato plants take up 2.0 g N m^{-2} kg^{-1} of fresh tomatoes. We determined a yield of 0.5 kg m^{-2} of the young tomato plants at the 5th of May 2015 (first harvest) and this was equal to 10 kg N ha^{-1} and this was equal for all the treatments. The only considerable nitrogen losses in these organic soils and in the open soilless culture system are nitrate leaching. It should be recognized that nitrogen uptake by the plants is a difficult parameter to estimate, since accurate estimates of both the total plant N and the total N supply are not easy to obtain (Hermanson et al. 2000). The accumulation of nitrogen by a crop is typically expressed on a per plant or per area (hectare) basis. Small areas and numbers of plants are sampled out of larger plots, and this was extrapolated to the larger area basis. In addition, only the aboveground crop mass (plant) was sampled because the roots are difficult to sample. In addition, the nitrogen content of the tomatoes was not analyzed, but estimated. All the supplied fertilizers and manures are 100% organic derived materials. Consequently, the supplied organic nitrogen was not considered for GBOF, SOILANIMAL and SOILPLANT in the supplied nitrogen, while it was considered for GBFISH, where ammonium and nitrate were used in the fertigation solution. As shown, by Hartz and Johnstone (2006) 60% of organic N of the blood meal (252 kg N ha^{-1}) had been mineralized within 2 weeks. For the malt sprouts (300 kg N ha^{-1}) Stadler et al. (2006b) determined a net mineralization of 70% after 35 days.

Table 6.1 Uptake and supply items of the considered balances of plant available N (profile: 0-10 cm soil profile); ANM: apparent net N mineralization; 2015 (time point 1= 11th of February 2015 – time point 2= 27th of July 2015 and time point 3 = 4th of November 2015).

	ANM (t1-t2)	**ANM (t2-t3)**	**ANM (t1-t3)**
N-Plant uptake	profile mineral N at t2 plant N uptake at t1 (10 kg ha^{-1})	profile mineral N at t3 plant N uptake	profile mineral N at t3 total N uptake
N-supply	profile mineral N at t1 N supply at start t1	profile mineral N at t2 N supplied at t2	profile mineral N at t1 total N supplied

The supplied organic derived nitrogen for GBOF mineralized within a few days for 80% (personal communication, Frayssinet). Consequently, part of the apparent net nitrogen mineralization comes

from the fertilizer used, i.e. 60–70% depending on the fertilizer and the rest is coming from the mineralization from the soil organic matter. Mineralization of the organic matter coming from the organic growing medium in GBOF and GBFISH is negligible.

2.5 Plant performance

The length of the plant was measured on a weekly basis with a measuring tape. Both the fresh and dry weight of the plants and nitrogen content were determined at the start (11/2/2015), the middle (27/7/2015) and at the end (4/11/2015) of the experiment. Tomatoes were harvested on a weekly basis or whenever necessary and cumulative yield (fresh weight) was determined.

2.6 PLFA analyses

Microorganisms were analyzed using the phospholipid fatty acid analysis (PLFA). Briefly, PLFAs were extracted from freeze-dried soil and growing media using a modified technique (Bligh and Dyer 1959) The PLFAs were determined using a procedure modified from Balser (2001), as fully described by Moeskops et al. (2010). To identify gram-positive bacteria, the sum of i14:0, i15:0, a15:0, i16:0, a16:0, i17:0 and a17:0 was used. The fatty acids cy17:0, cy17:0new, cy19:0 and cy19:0new were considered to be characteristic for gram-negative bacteria. The sum of 10Me16:0, 10Me17:0 and 10Me18:0 was regarded as a reliable indicator for the Actinomycetes. The fatty acid 18:2ω6c was used as signature fatty acid for fungi, and two alternative signature fatty acids for fungi were considered as well, *i.e.* 18:1ω9 and 18:3 ω3. The fatty acid 16:1ω5c was used as signature for arbuscular mycorrhizal fungi (AMF). Bacteria: fungi (B:F) ratios were calculated by dividing the sum of markers for gram-positive, gram-negative bacteria, 15:0 and 17:0 by the fungal marker 18:2ω6c. Samples for PLFA analyses were collected at 4 different time points (11/2/2015, 11 /3/2015, 27/7/2015, 4/11/2015).

2.7 DNA extraction

Total DNA was extracted from the growing medium samples using the Power Soil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), following the manufacturer's instructions. Five hundred milligrams were used from the bulk and 0.1 g from the rhizosphere. Concentration and quality of DNA were measured based on the absorbance at 260 and 280 nm in a Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.8 Illumina library generation

High-throughput amplicon sequencing of the V3 – V4 hypervariable region (Klindworth et al. 2012) was performed with the Illumina MiSeq platform according to the manufacturer's guidelines at LGC Genomics GmbH (Berlin, Germany). Contigs were created by merging paired-end reads based on the Phred quality score (of both reads) heuristic as described by Kozich et al. (2013) (Kozich et al. 2013) in Mothur (Schloss et al. 2009) (v.1.33.3). Subsequently, unique sequences were aligned to the mothur-reconstructed SILVA SEED alignment (v123). Sequences falling outside of the alignment space and with homopolymers exceeding those found within the SEED alignment were removed. Alignments were filtered to remove empty columns and again only unique sequences were retained. Next, sequences were pre-clustered together within a distance of 1 nucleotide per 100 nucleotides. These cleaned-up and preclustered sequences were checked for chimeras using Uchime v4.2.40 (Edgar 2010). Sequences were classified using the RDP trainset (Cole et al., 2007) version 9, removing those with Eukaryota,

Mitochondria or Chloroplast classification. The sequences were clustered into operational taxonomic units (OTUs) at 97% identity level with UPARSE (Edgar 2013) on default settings (v7.0.1001_i86linux32) via the sortbysize, cluster_otus, and usearch_global commands. Quality of the sequencing and post-processing pipeline was verified by incorporating mock samples (n = 12 species) in triplicate into the same sequencing run. A total of 868,162 of reads were obtained. After examining read counts, if any OTU was not classified up to genus level, the consensus sequence was blasted using the NCBI database to obtain the taxonomic classification. Singletons that remained unclassified were culled. Because of the over-dispersion in the OTU data, a zero-inflated count model was used to assess the effect of tomato cultivating system and time and the interactions between "tomato cultivating system" and time on each individual genus. Zero-inflated models explain the excess of zeros by modeling the data as a mixture of a Poisson distribution or a negative binomial distribution. When a zero count is observed, there is the zero-inflation probability, because the observation came from the always-zero distribution. When the underlying count distribution is a Poisson distribution, the model is called a zero-inflated Poisson distribution and if the count distribution is a negative binomial distribution, the mixture is called a zero-inflated negative binomial distribution. The final model was selected based on the Akaike Information Criterion (AIC). Differences among library size sample were accounted for with the offset option in proc GLIMMIX in SAS (Paschold et al. 2012). P values for each comparison were converted to q-values that were then used to identify differences in relative abundances of bacterial genera while controlling false discovery rate (FDR) at the 5% level (Storey 2015).

2.9 Multivariate statistical analysis

Differences in tomato cultivation systems and nitrogen dynamics were compared using a mixed model in SAS. P-values for Pearson correlation coefficients and regression coefficients were used to assess significant relations and significance was assumed at $P < 0.05$.

Multiple Factor Analysis (MFA) was employed to detect how the microbial community composition based on a PLFA analysis and bacterial abundance contributed to the differences between the four different tomato cultivating systems across time points. In addition, MFA was applied to the whole set of variables to assess the correlations among the chemical and microbiological variables based on the PLFA analysis and bacterial abundance detected in the four tomato cultivating systems.

The function MFA from the FactoMineR package (Lê et al. 2008) was performed in R. In addition, Multiple Factor Analysis (MFA) assisted to detect how the relative abundances of bacterial genera differed in growing media harboring either of the two plants. The function MFA from the FactoMineR package (Lê et al. 2008) was performed in R. Parametric bootstrapping was applied to construct confidence ellipses around the barycenter of the samples included on each covariate (time/fertilizer/plant), and thus visualize whether the bacterial abundances were significantly different among any of these categorical descriptors.

Richness, Fisher's diversity, Shannon, Simpson and inverse Simpson indices were calculated to assess alpha diversity within each sample. Pielou's index was used as indicator of evenness in the community. Differences in alpha diversity and evenness measures among treatments were compared using a repeated measures mixed model in SAS (version 9.4, SAS Institute, Cary, USA) with the four tomato cultivating systems as a fixed effect and time. Hence, the differences in the diversity measures could be attributed time or tomato cultivating system or to the interaction of the two factors.

Beta diversity estimates based on Chao and Bray-Curtis indices were used to examine dissimilarity and determine the impact of experimental factors on microbial community composition. Non-metrical multidimensional scaling nMDS (Supplementary figure 6.2) was employed to visualize the differences among samples, using the vegan package in R (Oksanen et al. 2007). Stratified permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was conducted to indicate the significance of each covariate (time, fertilizer and growing medium pre-treatment) on the microbial community of the bulk and rhizosphere. ANOVA was applied to reveal whether the distribution of the genera was different between plants (Oksanen et al. 2007).

3. Results

3.1 Evolution of chemical variables throughout the whole study period of four different tomato cultivating systems

The four different tomato cultivating systems differed significantly between each other (P<0.001) and are highly variable over time (P<0.001) (Table 6.2).

*Table 6.2: Average values of eleven different variables throughout the entire experimental period with standard error. n= 118. Differences in variables among treatments were compared using a repeated measures mixed model in SAS. Factors that differ more than a factor 2-3 are indicated in bold. * Chemical analysis are expressed as mg L^{-1} growing medium*

Variable	Treatment (trt)				p-value (trt)	P-value (time)
	GBOF	GBFISH	SOILANIMAL	SOILPLANT		
pH(H_2O)	6.1±0.04 [b]	5.1±0.04 [a]	6.8±0.05 [d]	6.6±0.05 [d]	<0.001	<0.001
EC (µS cm^{-1})	**596±34 [c]**	**883±34 [d]**	**243±37 [a]**	**364±40 [b]**	<0.001	<0.001
NO_3^--N (mg L^{-1*})	**14±12 [a]**	**332±12 [c]**	**23±13 [b]**	**25±14 [b]**	<0.001	<0.001
NH_4^+-N (mg L^{-1})	**40± [c]**	**12±2 [b]**	**1±2 [a]**	**3±2 [a]**	<0.001	<0.001
NO_3^--N/ NH_4^+-N ratio	0.35	27	23	8		
P (mg L^{-1})	**218±14 [b]**	**216±14 [b]**	**37±15 [a]**	**35±16 [a]**	<0.001	<0.001
K^+ (mg L^{-1})	**382±12 [c]**	**423±12 [d]**	**58±13 [a]**	**88±15 [b]**	<0.001	<0.001
Ca^{2+} (mg L^{-1})	1234±33 [b]	1173±34 [b]	937±36 [a]	1103±38 [b]	<0.001	<0.001
Mg^{2+} (mg L^{-1})	254±9 [c]	286±9 [d]	138±10 [a]	182±10 [b]	<0.001	<0.001
SO_4^{2-} (mg L^{-1})	740±40 [c]	708±40 [b]	448±44 [a]	799±4 [c]	<0.001	<0.001
Na^+ (mg L^{-1})	118± [c]	97±6 [b]	33±7 [a]	42±7 [a]	<0.001	<0.001
Cl^- (mg L^{-1})	**340±20 [c]**	**43±20 [b]**	**18±22 [a]**	**18±24 [a]**	**<0.001**	**<0.001**

Electrical conductivity, nitrate, ammonium, phosphorus, potassium, sodium and chloride differed more than factor 2-3 between each other. Differences in chemical composition among treatments were compared using a repeated measures mixed model in SAS (version 9.4, SAS Institute, Cary, USA) with the four tomato cultivating systems as a fixed effect and time as random effect.

3.2 Effect of tomato cultivation system on species richness, diversity and evenness of bacterial abundance over time

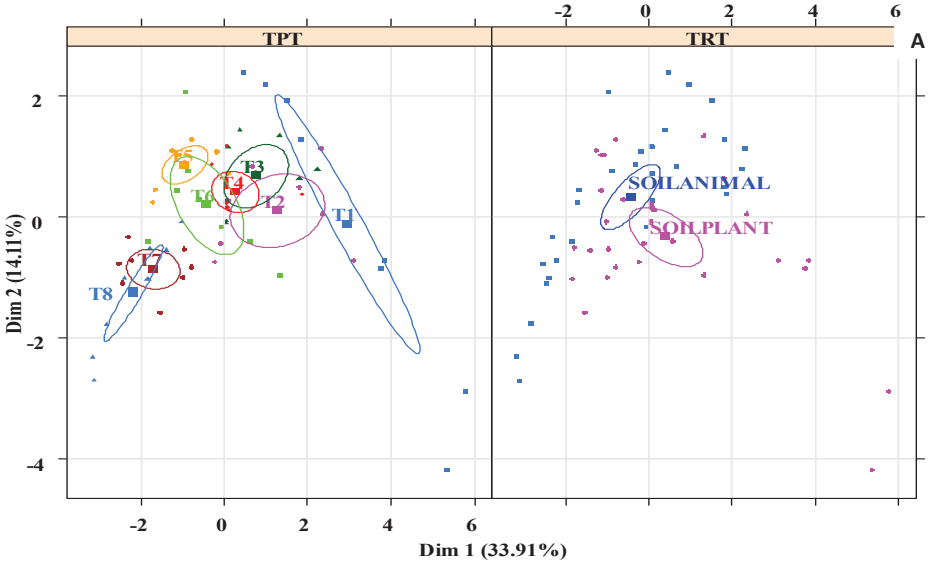
Table 6.3: Effect of tomato cultivation system (GBOF, GBFISH, SOILANIMAL and SOILPLANT) on species richness (total species), diversity (Shannon, Fisher's alpha, Simpson and Inverse Simpson indices), and evenness (Pielou's index) for all the eight time points (n=3). GBOF = organic growing medium in combination with organic fertilizer, GBFISH= organic growing medium with fish effluent, SOILANIMAL = organic soil with animal derived material as fertilizer (blood meal) and SOILPLANT= organic soil with plant derived material as fertilizer (malt sprouts). NS = not significant effect. Different superscripts indicate significantly different means. SEM= standard error of the mean.

Index	Time	tomato cultivation system (treatment)				SEM	Effect		
		GBOF	GBFISH	SOILANIMAL	SOILPLANT		treatment	Time	treatment* time interaction
Total species	T1	124.3 ^{a}	98.8 ^{a}	332.8 ^{b}	336.8 ^{b}	37.3	<0.0001	<0.0001	<0.0001
	T2	84.5 ^{ab}	52.8 ^{a}	413.5 ^{c}	116.0 ^{b}				
	T3	100.5 ^{a}	143.0 ^{b}	394.3 ^{c}	437.3 ^{d}				
	T4	295.0 ^{a}	205.3 ^{b}	412.0 ^{c}	413.5 ^{c}				
	T5	281.5 ^{a}	203.5 ^{b}	406.5 ^{c}	403.3 ^{c}				
	T6	265.5 ^{b}	105.5 ^{a}	321.5 ^{c}	382.3 ^{d}				
	T7	339.5 ^{b}	145.3 ^{a}	385.8 ^{c}	403.0 ^{c}				
	T8	179.8 ^{a}	223.3 ^{b}	151.0 ^{a}	409.0 ^{c}				
Pielou	T1	0.625 ^{a}	0.595 ^{a}	0.783 ^{b}	0.793 ^{b}	0.024	<0.0001	<0.0001	<0.0001
	T2	0.647 ^{a}	0.706 ^{a}	0.777 ^{b}	0.792 ^{b}				
	T3	0.810 ^{a}	0.713 ^{c}	0.772 ^{b}	0.760 ^{b}				
	T4	0.708 ^{a}	0.758 ^{a}	0.784 ^{b}	0.775 ^{b}				
	T5	0.750	0.760	0.768	0.772				
	T6	0.753	0.795	0.785	0.781				
	T7	0.736	0.726	0.785	0.776				
	T8	0.714 ^{a}	0.687 ^{b}	0.700 ^{c}	0.767 ^{a}				
Shannon	T1	2.778 ^{a}	2.563 ^{a}	4.544 ^{b}	4.609 ^{b}	0.211	<0.0001	<0.0001	<0.0001
	T2	2.709 ^{a}	2.794 ^{a}	4.678 ^{b}	3.764 ^{c}				
	T3	3.568 ^{a}	3.293 ^{a}	4.616 ^{b}	4.618 ^{b}				
	T4	4.022 ^{a}	3.952 ^{a}	4.718 ^{b}	4.665 ^{b}				
	T5	4.211 ^{a}	3.912 ^{a}	4.613 ^{b}	4.626 ^{b}				
	T6	4.062 ^{a}	3.631 ^{a}	4.484 ^{b}	4.640 ^{b}				
	T7	4.288 ^{a}	3.256 ^{b}	4.672 ^{c}	4.651 ^{c}				
	T8	3.602 ^{a}	3.647 ^{a}	3.302 ^{b}	4.613 ^{c}				
Simpson	T1	0.862 ^{a}	0.812 ^{a}	0.973	0.977	0.028	<0.0001	<0.0001	<0.0001
	T2	0.790 ^{a}	0.816 ^{a}	0.979	0.921				
	T3	0.935 ^{a}	0.891 ^{a}	0.978 ^{b}	0.979 ^{b}				
	T4	0.964	0.960	0.982	0.980				
	T5	0.969	0.928	0.976	0.976				
	T6	0.955	0.936	0.974	0.980				
	T7	0.963 ^{a}	0.858 ^{b}	0.979 ^{c}	0.979 ^{c}				
	T8	0.934	0.899	0.858	0.974				
Inverse Simpson	T1	7.427 ^{a}	6.049 ^{a}	38.127 ^{b}	44.223 ^{c}	4.884	<0.0001	<0.0001	<0.0001
	T2	7.891 ^{a}	6.564 ^{a}	48.304 ^{b}	14.460 ^{c}				
	T3	19.618 ^{a}	13.168 ^{a}	46.218 ^{b}	47.679 ^{b}				
	T4	27.817 ^{a}	26.136 ^{a}	55.706 ^{b}	49.539 ^{c}				
	T5	33.674 ^{a}	32.469 ^{a}	41.571 ^{b}	42.066 ^{b}				
	T6	33.669 ^{a}	21.518 ^{a}	41.184 ^{b}	50.692 ^{c}				
	T7	37.159 ^{a}	18.664 ^{b}	47.643 ^{b}	48.561 ^{c}				
	T8	16.600 ^{a}	19.257 ^{a}	15.655 ^{b}	38.384 ^{c}				
Fisher's	T1	26.966 ^{a}	22.582 ^{a}	63.810 ^{b}	63.979 ^{b}	4.640	<0.0001	<0.0001	<0.0001
	T2	23.416 ^{a}	22.138 ^{a}	68.101 ^{b}	51.262 ^{c}				
	T3	34.273 ^{a}	35.035 ^{a}	65.298 ^{b}	69.475 ^{b}				
	T4	47.982 ^{a}	48.614 ^{a}	68.776 ^{b}	66.327 ^{b}				
	T5	48.196 ^{a}	49.268 ^{a}	67.699 ^{b}	66.150 ^{b}				
	T6	50.323 ^{a}	37.039 ^{b}	62.389 ^{c}	67.027 ^{c}				
	T7	55.162 ^{a}	36.092 ^{b}	69.952 ^{b}	68.985 ^{b}				
	T8	37.651 ^{a}	45.136 ^{a}	35.967 ^{b}	67.726 ^{c}				

Table 6.3 showed a significant effect of the tomato cultivation system (*P<0.0001*), time (*P<0.0001*) and the interaction between tomato cultivation system and time *P*<0.0001) on species richness, diversity and evenness (Pielou's index). SOILANIMAL and SOILPLANT had significantly higher species richness (total species) in comparison to GBOF and GBFISH. Evenness showed the same tendency, showing a significant higher evenness for SOILANIMAL and SOILPLANT, however from time point 5 not differences were found between the tomato cultivation systems. Based on the diversity the calculated indices showed significant differences between SOILANIMAL, SOILPLANT and GBOF, GBFISH.

3.3 Microbial community composition based on bacterial abundance

Mean relative abundances between time points had homogenous variances, indicating that the samples were comparable. These results indicate whether there were differences in composition within treatments (GBOF, GBFISH, SOILANIMAL and SOILPLANT) or time points. Hence, the dispersion of genera across the four treatments was used as a measure of beta diversity. Treatment (*P*< 0.001), time (*P*<0.001) and the interaction between treatment and time (*P*<0.001) had a significant effect on the relative abundances of the bacterial genera. nMDS (Supplementary figure 6.2) was employed to visualize the distribution of bacterial genera among samples, using the vegan package in R (Oksanen et al. 2007) and showed that the community structure was significantly different as a result of treatment. Anova, indicated that the distances to the centroid of the counts of each genus present in samples across the four treatments were not equal indicating that the dispersion of the genera among treatments were significantly different (*P*<0.001). In addition, the dispersion of the genera among soil and soilless culture systems seemed to be quite comparable, hence, it was decided to create separate plots for the organic soil and for the soilless culture system.



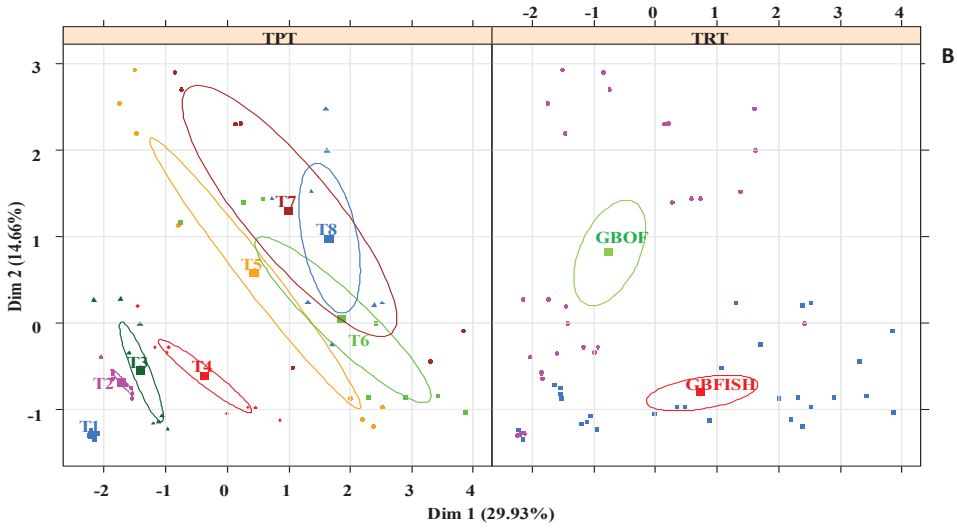


Figure 6.1: Multiple factor analysis in which 20 of the most significant factors were taken into account for the organic soil (A) and for the soilless culture system (B). TRT indicates the tomato cultivating system (GBOF= organic growing medium with organic fertilizers, GBFISH= organic growing medium with fish effluent, SOILANIMAL=organic soil that was fertilizer with animal-derived material and SOILPLANT= soil that was fertilized with plant-derived material). TRT = time point ("T1"= treatment at time point 1, "T2"= treatment at time point 2, "T3"= treatment at time point 3, "T4"= treatment at time point 4; T5"= treatment at time point 5; T6"= treatment at time point 6; T7"= treatment at time point 7; T8"= treatment at time point 8.). Circles indicate the 95% confidence interval.

Figure 6.1 showed the plots of the organic soil (A) and the soilless culture system (B). These plots indicated whether there were differences in composition within treatments (SOILANIMAL and SOILPLANT or GBOF and GBFISH) or time points. In the organic soil at the start of the cropping system (T1), the environment was significantly different and the variance (given by the size of the confidence ellipse) is reduced over time. The opposite happens in the soilless culture system. All samples were the same at the beginning, based on their physical and chemical variables measured and based on the bacterial populations present at the start of the experiment. When time passed by (T1 until T8), the variance was increased over time and GBOF and GBFISH become differentiated as a result of the fertilizer used. Multiple factor analyses (Figure 6.2, A) of the organic soil showed that plant length, pH, *Flavisolibacter*, phosphorus, chloride, ammonium, potassium, calcium, magnesium, sodium, electrical conductivity, nitrate, sulphate, *Desulfotomaculum*, *Solirubrobacter*, *Dehalococcoides*, *Bythopirellula*, *Steroidobacter*, *Litorilinea*, *Nonomuraea* are the 20 most significant factors used to discriminate between SOILANIMAL and SOILPLANT. The first dimension (33.9% of variance) of the organic soil (A) is positively correlated with t1 (P<0.001), and t2 (P=0.04) and negatively correlated with t7 (P=0.006) and t8 (P=0.0003), whereas the second dimension (14.1% of variance) is positively correlated with SOILANIMAL (P<0.03), and t5 (P=0.03) and negatively correlated with t7 (P=0.03), SOILPLANT (P=0.003) and t8 (P=0.001). The third dimension of the organic soil (A) is positively correlated with t5 (P<0.01) and negatively correlated with t2 (P< 0.0001), whereas the fourth dimension is positively correlated

with SOILANIMAL (P<0.001), and t1 (P=0.04) and negatively correlated with t6 (P<0.001) and SOILPLANT (P<0.0001).

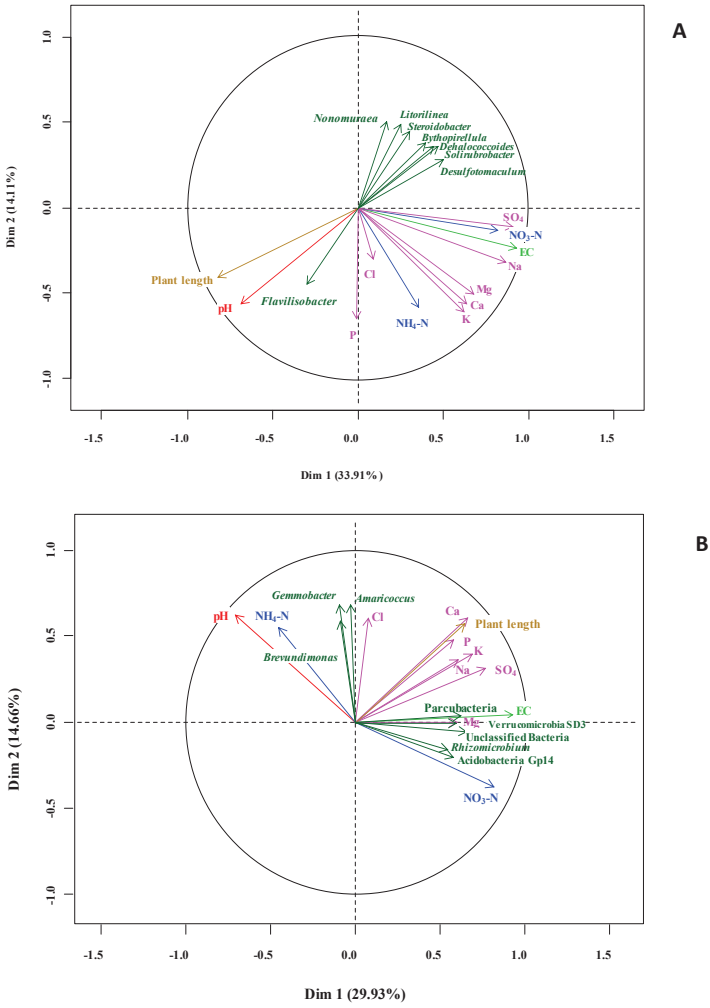


Figure 6.2: Plane of the first two dimensions (Dim1 and Dim 2) from a MFA in which 20 of the most significant factors were used to discriminate between the different tomato cultivation systems. The first dimension of the MFA for the organic soil (A) describes the differences between T1 and T2 (positively correlated) and T7, T8 (negatively correlated), while the second dimension describes the differences between SOILANIMAL and T5 (positively correlated) and SOILPLANT, T7, T8 (negatively correlated). The first dimension of the MFA for the soilless culture system (B) describes the differences between GBFISH, T6 and T8 (positively correlated) and GBOF, T1 and T2 (negatively correlated), while the second dimension describes the differences between GBOF, T7, T8 (positively correlated) and GBFISH, T1 (negatively correlated).

The fifth dimension of the organic soil (A) is positively correlated with t3 (*P*=0.002), and t6 (*P*=0.03) and negatively correlated with t2 (*P*=0.03) and t8 (*P*=0.001). Multiple factor analyses (Figure 6.2, B) of the soilless culture system showed that nitrate, *Acidobacteria Gp 14*, *Rhizomicrobium*, *Unclassified bacteria*, *Verrucomicrobia SD3*, magnesium, electrical conductivity, *Parcubacteria*, sulphate, sodium, potassium, phosphorus, plant length, calcium, chloride, *Amaricoccus*, *Gemmobacter*, ammonium, *Brevundimonas*, pH are the 20 most significant factors used to discriminate between GBOF and GBFISH.

The first dimension (29.9% of variance) of the soilless culture system (B) is positively correlated with GBFISH (*P*=0.001), t6 (*P*=0.001), t8 (*P*=0.005) and negatively correlated with t3 (*P*=0.02), t2 (*P*=0.001), t1 (*P*=0.002) and GBOF (*P*<0.001), whereas the second dimension (14.7% of variance) is positively correlated with GBOF (*P*<0.001), t7 (*P*=0.004) and t8 (*P*=0.002) and negatively correlated with t1 (*P*=0.009) and GBFISH (*P*<0.001). The third dimension of the soilless culture system (B) is positively correlated with t8 (*P*<0.03) and negatively correlated with t6 (*P*< 0.002), whereas the fourth dimension is positively correlated with t5 (*P*<0.001), and t6 (*P*=0.01) and negatively correlated with t8 (*P*<0.001). The fifth dimension is positively correlated with GBFISH (*P*=0.02), and t7 (*P*=0.03) and negatively correlated with GBOF (*P*=0.02) and t6 (*P*=0.004).

3.4 Microbial community composition based on PLFA

Combinations of organic soil with plant and animal-derived material and organic growing medium with fish effluent and organic fertilizer differed in its characteristics throughout the experimental period. Eleven soil and growing media characteristics (pH(H_2O), EC, nitrate, ammonium, phosphorus, potassium, calcium, magnesium, sulphate, sodium and chloride) and eight microbial characteristics (gram-positive, gram-negative bacteria, 18:1, 18:2 and 18:3 fungi, Actinomycetes, arbuscular mycorrhizal fungi and protozoa) were analyzed together in a multiple factor analysis (MFA, Supplementary figure 6.3 and Supplementary figure 6.4).

Regarding to the four different tomato cultivation systems, the first two dimensions (Supplementary table 6.5) accounted for 60.3% of the total variance. The first dimension describes GBOF (*P*=0.003) and GBFISH (*P*=0.015) (Supplementary figure 6.3, Supplementary figure 6.4 and Supplementary table 6.5), whereas the second dimension 2 is described by SOILPLANT (*P*=0.017) and time point 4 (*P*=0.014). Nitrate, phosphorus, potassium, calcium, magnesium, sulphate, sodium and chloride are significantly positively correlated (*P*< 0.05), while Actinomycetes, bacteria and fungi 18:2 ratio, and gram-positive bacteria and pH(H_2O) were significantly negatively correlated (*P*<0.05) with dimension 1. Arbuscular mycorrhizal fungi, gram-negative bacteria, calcium, sulphate, phosphorus, gram-positive bacteria, pH(H_2O), sodium and chloride were significantly positively correlated and ammonium, Actinomycetes, fungi 18:2 were significantly negatively correlated with dimension 2 (*P*< 0.05). Dimension 3 describes GBOF (*P*< 0.001) and dimension 4 is described by SOILANIMAL (*P*=0.005).

The soilless culture systems GBOF and GBFISH, however, showed a positive correlation with protozoa and the fungal FAME marker 18:2 and 18:3 and it was negatively correlated with the gram-positive bacteria and the Actinomycetes. The use of blood meal and malt sprouts in the organic soil was positively correlated to AMF, the gram-negative and gram-positive bacteria. Fungal FAME marker 18:1, 18:2 and 18:3 was negatively affected by the application of blood meal and malt sprouts. These results indicated increased disturbance caused by fertilizer incorporation, *i.e.* blood meal and malt sprouts and soil cultivation.

Arbuscular mycorrhizal fungi (AMF), gram-negative and gram-positive bacteria are positively correlated with SOILANIMAL and SOILPLANT. Arbuscular mycorrhizal fungi (AMF) formation may influence microbial communities in the rhizosphere, through variations in root exudates and translocation of C to the soil environment in the form of hyphal exudates (Gahan and Schmalenberger 2014). Actinomycetes and AMF are important soil quality indicators (Bending et al. 2004), however only AMF was positively stimulated in the organic soil in our experiment.

3.5 Electrical conductivity, pH(H_2O) and ammonium and nitrate concentrations in the soil and soilless culture system

Evolution of the electrical conductivity, pH(H_2O) and nitrate and ammonium concentration (Figure 6.3) of the four different treatment was followed over time (Supplementary table 6.5). The organic soil fertilized with organic fertilizer or animal-derived material (243 ± 111 µS cm^{-1}) or plant-derived material (344 ± 192 µS cm^{-1}) showed the lowest average electrical conductivity and it decreased over time. The soilless culture system in combination with organic growing medium showed higher values for the electrical conductivity (Figure 6.3, C) (GBOF=551 ± 323 µS cm^{-1} and GBFISH =905 ± 614 µS.cm^{-1}) and it increased over time. The pH(H_2O) of the soil increased over time for SOILANIMAL from 6.3 to 7.3 and SOILPLANT from 6.6 to 6.9, while the pH(H_2O) in the organic growing medium was very dynamic and fluctuated over time (Figure 6.3, D).

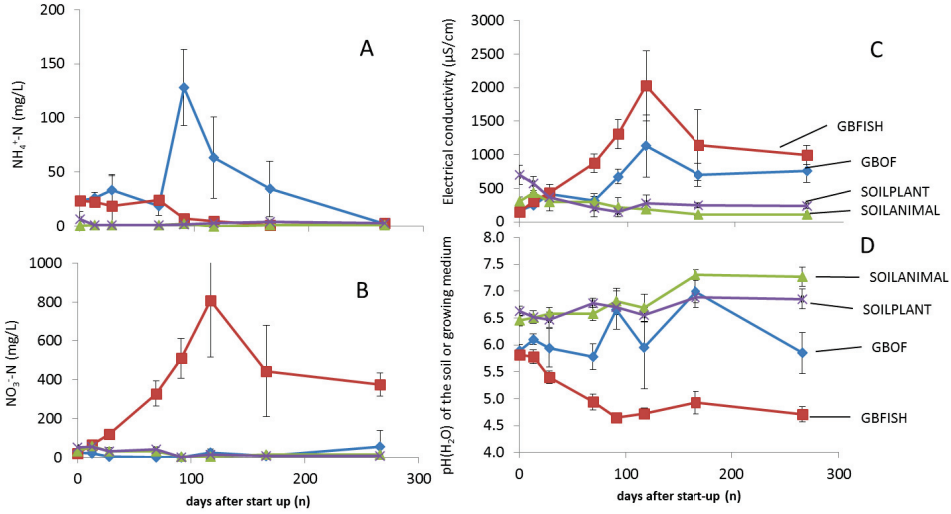


Figure 6.3: Evolution of the ammonium concentration (A), nitrate concentration (B), the electrical conductivity (C) and pH(H_2O) (D) in the growing medium, i.e. GBOF and GBFISH and the organic soil, i.e. SOILANIMAL and SOILPLANT during the whole experimental period which lasted 266 days.

The most important factor influencing the root induced changes in the rhizosphere and the bulk zone is the uptake of nutrients, coupled with H^+ transport in plants. Plants have the ability to change the rhizosphere acidity by releasing H^+ or OH^- to compensate for an unbalanced cation–anion uptake at the soil–root interface (Hinsinger et al. 2003; Riley and Barber 1969). The pH(H_2O) of GBFISH dropped

between days 13 and 91 from 5.7 to 4.6 indicating an increased uptake of cations, such as potassium and ammonium. Indeed, we found increased amount of ammonium in GBFISH until days 69. Tomato plants have an increased demand for potassium in their generative growth stage, resulting in increased release of H^+ ions. This event occurred in the GBFISH treatment. GBOF, however, showed the highest ammonium concentration (41.2 ±39.1 mg NH_4^+-N L^{-1}), while GBFISH had lower concentrations (13.1 ±10.0 mg NH_4^+-N L^{-1}, Figure 6.3 A). Nitrate concentration was the highest for GBFISH with 333.7±263.6 mg NO_3^--N L^{-1}, while for the other treatment the nitrate concentration was up to 10 times lower (Figure 6.3 B).

Supplementary table 6.1 shows the mineral nitrogen content in the organic soil in the 0-10 top soil layer. A bulk density of 1.25 t ha^{-1} (Vlaamse zandstreek) was assumed to calculate the nitrogen content per ha (Arthur et al. 2011). The nitrate content determined based on an ammonium lactate extract decreased over time in SOILANIMAL and SOILPLANT from 243 kg NO_3^--N ha^{-1} and 196 kg NO_3^--N ha^{-1}, respectively to approximately 10 kg NO_3^--N ha^{-1}. The ammonium concentration remained stable and was on average 6 kg NH_4^+-N ha^{-1}.

3.6 Estimation of the nitrogen dynamics

Table 6.4: N supply, Nmin (0-10 cm), N taken up by the plant and apparent net N mineralization (ANM) (kg ha^{-1}); numbers are estimates for the different three different time point in 2015 (time point 1= 11th of February 2015 – time point 2= 27th of July 2015 and time point 3 = 4th of November 2015).

Treatment	N-Supply (kg ha^{-1})			Nmin (kg ha^{-1})			N-Plant uptake (kg ha^{-1})			ANM (kg ha^{-1})		
	t1-t2	t2-t3	t1-t3	t1	t2	t3	t1-t2	t2-t3	t1-t3	t1-t2	t2-t3	t1-t3
GBOF	0	0	0	1	4	5	324	168	482	327	169	486
GBFISH	2436	1428	3864	1	40	34	397	202	589	-2000	-1440	-3242
SOILANIMAL	0	0	0	244	21	12	309	203	502	86	194	270
SOILPLANT	0	0	0	203	33	12	307	232	529	131	211	338

The four tomato cultivating systems differed from each other with respect to nitrogen dynamics, i.e. supplied nitrogen, mineral nitrogen, the nitrogen taken up by the plant and the apparent net nitrogen mineralization (Table 6.4). Based on the estimated nitrogen balance, a high apparent net N mineralization occurred with GBOF, SOILANIMAL and SOILPLANT and negative rates, i.e. apparent net N immobilization, occurred in combination with GBFISH. GBOF showed the highest apparent

net nitrogen mineralization compared to SOILPLANT and SOILANIMAL. The highest plant nitrogen uptake was found in combination with GBFISH, followed by SOILPLANT, SOILANIMAL and finally GBOF.

3.7 Plant performance (growth and yield)

Plant length was followed during 70 days after the start of the experiment. Plants in GBOF were 12-16% taller in comparison with the GBFISH, SOILANIMAL and SOILPLANT. The average growth rate for GBOF, GBFISH, SOILANIMAL and SOILPLANT was 3.74 cm d^{-1}, 3.27 cm d^{-1}, 3.27 cm d^{-1} and 3.1 cm d^{-1}.

Table 6.5: Overview of the yield and the quality of the tomatoes (red tomatoes, green tomatoes and tomatoes with blossom end rot (BER)) for four different tomato cultivating systems (GBOF, GBFISH, SOILANIMAL and SOILPLANT)

Object	Total yield (kg m^{-2})	Yield red tomatoes (g $tomato^{-1}$)	Red tomatoes	Green tomatoes	Tomatoes with BER
			%	%	%
GBOF	22.378	82.1	82.4	14.8	2.8
GBFISH	27.840	85.8	84.4	14.7	0.9
SOILANIMAL	22.501	85.8	86.2	13.8	0
SOILPLANT	24.127	93.5	86.8	13.2	0

Yield of the tomato plants in the four different cultivation systems (GBOF, GBFISH, SOILANIMAL and SOILPLANT) was followed over time. The fresh weight of four randomly chosen plants was measured at the end of the experiment (4/11/2015). The average fresh weight for GBOF, GBFISH, SOILANIMAL and SOILPLANT was 1.54 ± 0.34 kg $plant^{-1}$, 1.16 ± 0.21 kg $plant^{-1}$, 1.54 ± 0.39 kg $plant^{-1}$ and 1.77 ± 0.18 kg $plant^{-1}$ (Table 6.5 and Table 6.6). Figure 6.4 shows that the cumulative yield of the soilless culture system in combination with the organic growing medium and the inorganic fertilizer (GBFISH) resulted in 15-17% higher yields in comparison with the three other cultivation systems. SOILANIMAL produced 22.5 kg m^{-2}, SOILPLANT, 24.1 kg m^{-2}, GBOF produced, 23.8 kg m^{-2} and GBFISH produced 27.8 kg m^{-2}. Cumulative yield of GBOF was equal to GBFISH until 117 days after plantation.

Afterwards, cumulative yield dropped and followed the same trend as the two fertilizers in combination with organic soil, *i.e.* SOILANIMAL and SOILPLANT. This decrease in yield of tomatoes at the higher N-application rates can explain the increasing amounts of fruits with blossom end rot (BER). BER infection was especially pronounced in the GBOF treatment, where the highest ammonium concentration of 140 mg NH_4^+-N L^{-1} were found. The decrease in total nitrogen content of the plants seems to be more pronounced in combination with GBOF. BER is caused by insufficient supply of calcium (Ca^{2+}) into the apical part of the fruit, among other factors, which has been caused by transpiration problems (Heeb et al. 2005a). This insufficient supply of calcium (Ca^{2+}) was counteracted in our experiment by extra doses of calcium for the GBOF treatment, which resulted in plant recovery from this severe stress (Sandoval-Villa et al. 2001). In addition, the use of organic fertilizer in GBOF resulted in a change of the shape of the tomatoes varying from round shaped until egg shaped

tomatoes (Figure 6.4). Navarro et al. (2005), showed that treatments with ammonium changed the shape of the tomatoes, validating our results.

Table 6.6: Overview of the total number tomatoes and the distribution in percentage between loose and tomatoes per vine for the four different tomato cultivating systems (GBOF, GBFISH, SOILANIMAL and SOILPLANT)

Object	Tomatoes (number of tomatoes m^{-2})	Loose tomatoes	2 tomatoes per vine	3 tomatoes per vine	4 tomatoes per vine	5 tomatoes per vine	6 tomatoes per vine	7 tomatoes per vine
		%	%	%	%	%	%	%
GBOF	239	2,1	6,4	17,0	34,0	27,7	8,2	4,6
GBFISH	274	0,8	5,2	12,5	22,7	35,8	15,2	7,8
SOILANIMAL	226	3,3	7,2	12,7	24,7	35,0	12,7	4,4
SOILPLANT	224	4,4	5,0	15,6	22,4	37,2	11,9	3,6

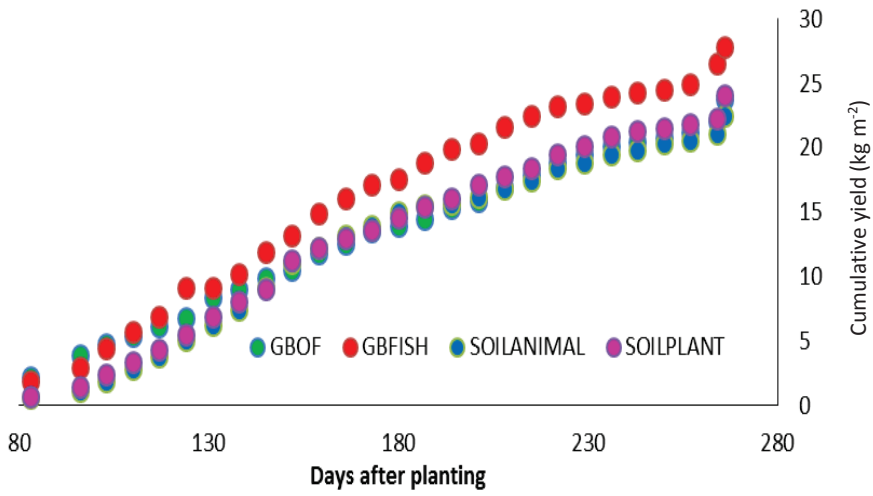


Figure 6.4: Evolution of the cumulative yield of the four different cultivation systems for tomatoes. GBOF: organic growing medium in combination with organic fertilizer, GBFISH= organic growing medium in combination with fish effluent, SOILANIMAL: organic soil fertilized with animal-derived material and SOILPLANT organic soil fertilized with plant-derived material.

4. Discussion

Given that soil and soilless culture systems in combination with organic growing media harbor distinctly different microbial communities and given that nitrogen fertilization, has effects on the microbial communities composition, we hypothesized that differences in nitrogen dynamics of the applied fertilizers will influence the microbial community composition over time.

4.1 Biodiversity, species richness, and evenness in relation to the four tomato cultivation systems

A total of 11 chemical variables were monitored for the four different tomato cultivating systems. No major plant growth anomalies were found, except for GBOF with the highest nitrogen supply rate. We found a significant effect tomato cultivating system (P<0.0001) and time (P<0.001) on species richness. Our MFA analysis based on the PLFA results and based on high throughput sequencing of the 16S rRNA gene and chemical factors, indicate that the dispersion of the genera among treatments is significantly different (*P<0.001*). Indeed, the diversity of microbial communities associated with the soil or soilless culture system are directly influenced by the physical and chemical properties of the soil. It must be considered that four different tomato cultivating systems supplemented with different fertilizers were compared with each other, making it impossible to estimate the separate effect of soil type or growing medium or fertilizer used on the microbial community composition. However, SOILANIMAL and SOILPLANT showed similar microbial community composition, i.e. richness, evenness and GBOF and GBFISH also showed a more similar microbial community composition indicating a potential soil or growing medium effect. On the other hand, within the organic soil and soilless culture system the microbial community composition seemed to be different depending on the fertilizer used (animal or plant based nutrients or organic versus inorganic). Schutter et al. (2001) indicated that soil microbial compositions were influenced by soil type than by farm management. As shown by Bossio and Scow (1998) the source of nutrients used, such as inorganic versus decomposed plant material, had a larger effect on microbial communities than land management systems (organic, low-input, or conventional).

Our analysis clearly showed that the bacterial species richness is increased over time. On average, the increase in species richness was about 18% for SOILPLANT and 20% for SOILANIMAL compared to the soilless culture system, where the species richness was increased by 14% for GBFISH and 21% for GBOF. Thus, our analysis supported the proposal that organic fertilizer in soilless culture systems and organic soils increase the species richness compared to the use of inorganic fertilizers. Thus, the original suggestions by Paoletti et al. (1992) and Schönning and Richardsdotter-Dirke (1996) that organic farming enhances biodiversity is supported by our analysis and in addition this is also true for soilless culture systems in combination with organic fertilizers.

We found a significant effect tomato cultivating system (P<0.000) and time (P<0.001) on species evenness. Further the diversity of bacterial communities was consistently higher in organic soils compared to soilless culture system, but the differences between the different tomato cultivation systems decreased over time. The species diversity of the bacterial communities was higher in SOILPLANT compared to the other three tomato cultivating systems. From time point 5 on no significant differences were found between the different tomato cultivating systems, whereas at time point 8 GBOF and SOILPLANT had the same score for evenness. On average, the increase in species evenness was about 0.2% for SOILPLANT and 0.6% for SOILANIMAL compared with the soilless culture system, where the species evenness was increased by 1.2% for GBFISH and 1.1% for GBOF. Indeed, we also found a time effect for evenness. In many cases, changes in evenness occur with little or no

changes in species richness, and this points to the importance of evenness as a component of diversity (Wilsey and Potvin 2000). As stated by Wittebolle (2009) unevenness could block the rapid response of a community to a particular stress if the dominant species are not resistant to this stress. It is reported that even communities can recover their function more easily, provided with sufficient time (Wittebolle 2009).

Organic management practices commonly lead to increased soil microbial biomass, increased microbial activity, and increased microbial species richness and diversity when compared to conventional farming (van Diepeningen et al. 2006). Ma et al. (2016) found no significant difference in bacterial community structures between organically and conventionally managed agricultural soils (Semenov, et al., 2008; Ma et al., 2012b). The study of Ma et al. (2016) as well as others suggest that differences in agricultural management may not be well reflected in bacterial diversity indices, but in overall bacterial community structure. The higher similarity in soil properties of the organic soil and the organic growing medium may also explain that no major differences in bacterial community structure were found between SOILANIMAL and SOILPLANT and GBOF and GBFISH, respectively, indicating that soil or growing medium are major discriminants of the microbial community composition. It has been shown that the composition, and in some cases diversity, of soil bacterial communities is often strongly correlated with soil pH (Rousk et al. 2010) and nitrogen fertilization (Martinez and Abad 1992).

MFA analysis showed that the soilless culture community of GBOF was positively correlated to the abiotic variables, notably ammonium concentration, pH(H_2O) and the chloride concentration, while community of GBFISH was positively correlated to the electrical conductivity, nitrate and magnesium. Ammonium is a by-product of the microbial decomposition of the organic fertilizer in GBOF and has strong impact on the pH of the growing medium and chloride is often found in high concentrations in organic fertilizers, while nitrate was the main nitrogen form used in GBFISH. This pattern was not that clear in combination with SOILANIMAL and SOILPLANT. The abiotic variables, notably nitrate and soil pH were the most significant factors determining the environmental niche of SOILANIMAL bacterial community, indicating nitrifying activity in the soil. Phosphorus, sodium, magnesium, calcium, potassium, chloride, pH and ammonium were the most significant factors of the SOILPLANT microbial community, indicating a higher ammonification rate than nitrification rate, as nitrate concentrations were the same for SOILANIMAL and SOILPLANT.

In this study, it was clear that community structure, based on PLFA results and bacterial abundance the organic soil and the soilless culture system were distinctly and consistently different over time. This could be attributed to differences in chemical characteristics of the four tomato cultivating systems.

4.2 Beta-diversity estimates of the four tomato cultivating systems shows different variability over time.

We hypothesized that beta-diversity estimates based on Chao and Bray-Curtis indices would vary differently between tomato cultivating systems after fertilizer application (plant derived or animal derived in the organic soil and inorganic or organic fertigation in the soilless culture system) over time. In the organic soil, the fertilizers are added as base dressing, while in the soilless culture the fertilizers are applied by daily fertigation based on solar irradiation. Figure 6.1 shows that all samples of GB were

the same at the beginning, based on their physical and chemical variables measured and based on the bacterial populations. MFA results indicate that they become increasingly differentiated over time according to the fertigation systems used, i.e. GBOF versus GBFISH. The opposite happens in the organic soil with an initial application of malt sprouts or blood meal at the start of the crop. At the beginning of the crop (T1), the environment is different because of the fertilizer application, i.e. an animal or a plant derived fertilizer and the variance (given by the size of the confidence ellipse) is reduced over time. Management practices, including nitrogen fertilization, impact the structure of soil microbial communities (Ramirez et al. 2010). Mentioned study showed by examining shifts in bacterial communities across contrasting ecosystem types (conventional versus organic) that bacterial community structure was highly sensible to N additions. Ramirez et al. (2010) suggested that bacterial communities across these gradients are more structured by N and/or soil carbon availability than by soil pH associated with the elevated nitrogen inputs. In addition, we found significantly different time shifts of the microbial community between the four tomato cultivating systems.

These results suggest that the variability over time needs to be carefully assessed when comparing microbial diversity across tomato cultivating systems.

4.3 The four tomato cultivating systems in relation to N dynamics and plant performance

The average fresh weight of the plant for GBOF, GBFISH, SOILANIMAL and SOILPLANT was 1.54 ± 0.34 kg plant^{-1}, 1.16 ± 0.21 kg plant^{-1}, 1.54 ± 0.39 kg plant^{-1} and 1.77 ± 0.18 kg plant^{-1}. The yield per surface unit for GBOF (23.8 kg m^{-2}) and SOILANIMAL (22.5 kg m^{-2}) and SOILPLANT (24.1 kg m^{-2}) was similar for the three treatments; GBFISH produced the highest amount of tomatoes, 27.8 kg m^{-2}.

The estimated total nitrogen uptake was the highest with GBFISH, i.e. 589 kg N ha^{-1} and SOILPLANT, i.e. 529 kg N ha^{-1}, while the N uptake by the plants in combination with GBOF was 482 kg N ha^{-1} and 502 kg N ha^{-1} for SOILANIMAL. Differences in nitrogen uptake were the biggest between time point 1 and time point 2, while between the 2nd and the 3rd time point the highest nitrogen uptake was found for SOILPLANT (232 kg N ha^{-1}), followed GBFISH (202 kg N ha^{-1}) and SOILANIMAL (203 kg N ha^{-1}) and finally GBOF 168 kg N ha^{-1}. Apparent net mineralization was positive for GBOF, SOILANIMAL and SOILPLANT, while this was negative for GBFISH. Based on the balance results, high apparent net N mineralization rates appeared for the treatments with organic derived fertilizer, i.e. GBOF, SOILANIMAL and SOILPLANT and negative rates (i.e. apparent net N immobilization) for GBFISH, i.e. mineral fertigation, throughout the entire growth season. A negative apparent net nitrogen mineralization for GBFISH for all the time point shows that this excess mineral N was either immobilized by the organic growing medium, which is possible for the ammonium and less likely for nitrate. Another possibility is the loss of nitrogen through the drainage solution, it was estimated at 1013 kg N ha^{-1}, which is very likely. The microbial community associated with the growing medium may also immobilize mineral N, however there is stronger competition for ammonium than for nitrate (Hodge et al. 2000). The N uptake in the root system may also be responsible for the immobilization effect. In these cases where the roots are not measured, the total plant N (shoots + roots) is typically under-represented by 5-15% (Hermanson et al. 2000). The sum of the initial soil mineral N amount and the mineral N input by fertilization or fertigation plus the apparent net N mineralization is a good estimate for the plant available nitrogen. This plant available nitrogen can be taken up by the plant considering the residual soil mineral N. The contribution of apparent net N mineralization to plant available nitrogen was 98.9 % in GBOF, 95.7% in SOILANIMAL and 96.6% in SOILPLANT.

From the experimental setup it is clear that we have different forms (organic nitrogen, ammonium and nitrate – nitrite is not considered) and concentrations of nitrogen in the four tomato cultivating systems. Plants can assimilate these different kind of nitrogen forms. Mineralization rates are not equal for the different organic fertilizers, such as the blood meal, malt spouts and the organic fertilizers used in combination with GBOF. In addition, mineralization first releases ammonium, that is then converted in nitrate during nitrification, so the abundance of nitrate depends on both the abundance of ammonium and the ammonia and nitrite oxidation rate (Boudsocq et al. 2012). Our results show a higher ammonium concentration in combination with GBOF, indicating a higher ammonification rate or a lower nitrification rate. From an energetic point of view, ammonium uptake and assimilation are less costly than nitrate uptake and assimilation, indicating a competitive advantage for plants with a high ammonium absorption capacity. Ammonium can cause severe toxicity symptoms (Britto and Kronzucker 2002). This ammonium toxicity may jeopardize the energetic advantage of taking up ammonium rather than nitrate. Plants need large amounts of potassium, calcium, magnesium, and other cations besides nitrogen. Increased incidence of blossom end rot indicates indeed increased ammonium uptake by the tomato plant in combination with GBOF. Accumulation of ammonium is most likely the result of a too high organic nitrogen supply rate compared to the ammonia oxidation rate, i.e. 163 mg N kg^{-1} growing medium d^{-1} compared to the potential ammonia oxidation rate (**Chapter 3**) of 83 mg NH_4^+-N kg^{-1} growing medium d^{-1}. Furthermore, ammonium is known for its abiotic immobilization, while nitrate is highly mobile and can lead to leaching losses. These physical limitations, energetic costs and competition with the soil microorganisms make these systems highly dynamic and almost unpredictable. It seems that the highest tomato yields and the lowest nitrogen uptake by the plants occur when the nitrate/ammonium ratio is 27 soilless culture system. According to Heeb et al. (2005a) modern soilless culture systems have a nitrate over ammonium ratio set at 10:1 or 18:1 and ammonium levels not exceeding 14 mg L^{-1}. The optimal growth of tomato roots occurs in soils with a ratio of nitrate to ammonium of 3:1 and is inhibited if the concentration of ammonium is too high (Glass and Siddiqi 1995; Haynes and Goh 1978). We had a ratio of 23 for SOILANIMAL and 8 for SOILPLANT, which is close to the optimal ratio of 3:1.

The yield per surface unit for GBOF and SOILANIMAL and SOILPLANT was similar for the three treatments. However, when yield is calculated per unit of volume, we found final cumulative yield of 3.1 kg tomatoes L^{-1} of growing medium and 2.8 kg tomatoes L^{-1} of growing medium for GBFISH and GBOF. The organic soil (SOILANIMAL and SOILPLANT), however, produced approximately 0.5 kg tomatoes L^{-1} soil. Soilless culture systems possess a finite buffer capacity regarding water and fertilizer supply, as well as pH-value of the nutritive solution, due to relatively small and restrictive root areas (Gruda 2008). Consequently, insufficient water supply and high organic nitrogen supply rates will derive in nutrient imbalance and further induce blossom end-rot (BER) of glasshouse tomatoes.

In this study, nitrogen conversions in the soil are dynamic and the contribution of the apparent nitrogen mineralization to plant available nitrogen is substantial.

5. Conclusions

We investigated soil and soilless microbial communities and their structure and dynamics and N dynamics and plant performance. In this work, the soil and soilless microbial communities, N conversions and plant performance were studied in four contrasting tomato cultivating systems and systematically compared over an entire period of 10 months. Organic soil and soilless systems were all

placed under optimal growing conditions for the same crop to compare microbial community ecology and nitrogen conversions and plant performance. We showed that:

The community structure of the organic soil and the soilless culture system were distinctly and consistently different over time and this could be attributed to differences in chemical characteristics of the four tomato cultivating systems.

The increase in species richness was around 18% for SOILPLANT and 20% for SOILANIMAL compared with the soilless culture system, where the species richness was increased by 14% for GBFISH and 21% for GBOF.

The increase in species evenness was around 0.2% for SOILPLANT and 0.6% for SOILANIMAL compared with the soilless culture system, where the species evenness was increased by 1.2% for GBFISH and 1.1% for GBOF.

The variability over time needs to be carefully assessed when comparing microbial diversity across tomato cultivating systems and the temporal patterns in microbial community structure diversity is affected because of different fertilization strategies

Nitrogen conversions in the soil and soilless culture systems are dynamic. The sum of the initial soil and soilless mineral N and the mineral N input by fertilization or fertigation plus the apparent net N mineralization is a good estimate for the plant available nitrogen.

These finding provide a first basis for understanding how soil and soilless communities differ from each other. Combining next-generation sequencing and chemical characteristics can provide insight into the factors influencing the overall diversity of soil and soilless microbial communities.

6. Acknowledgements

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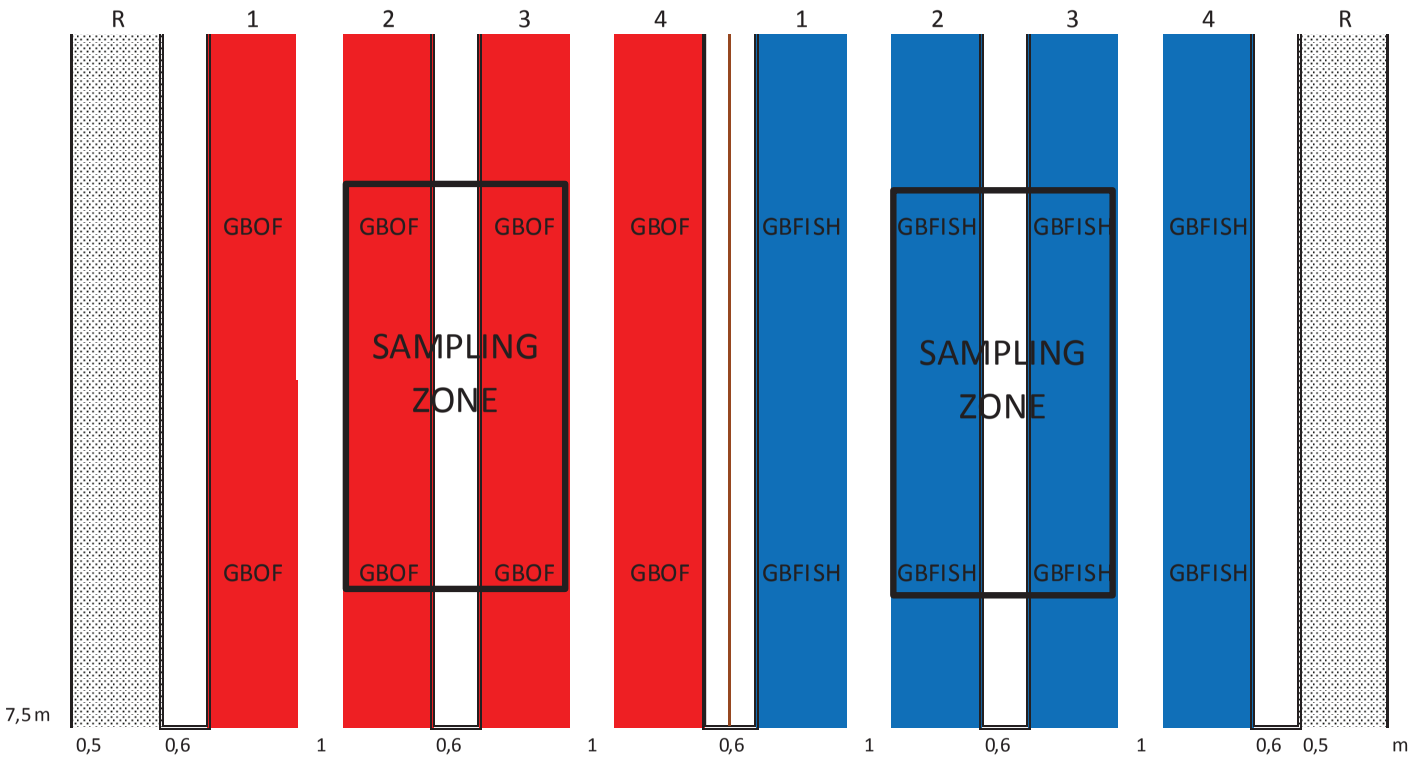
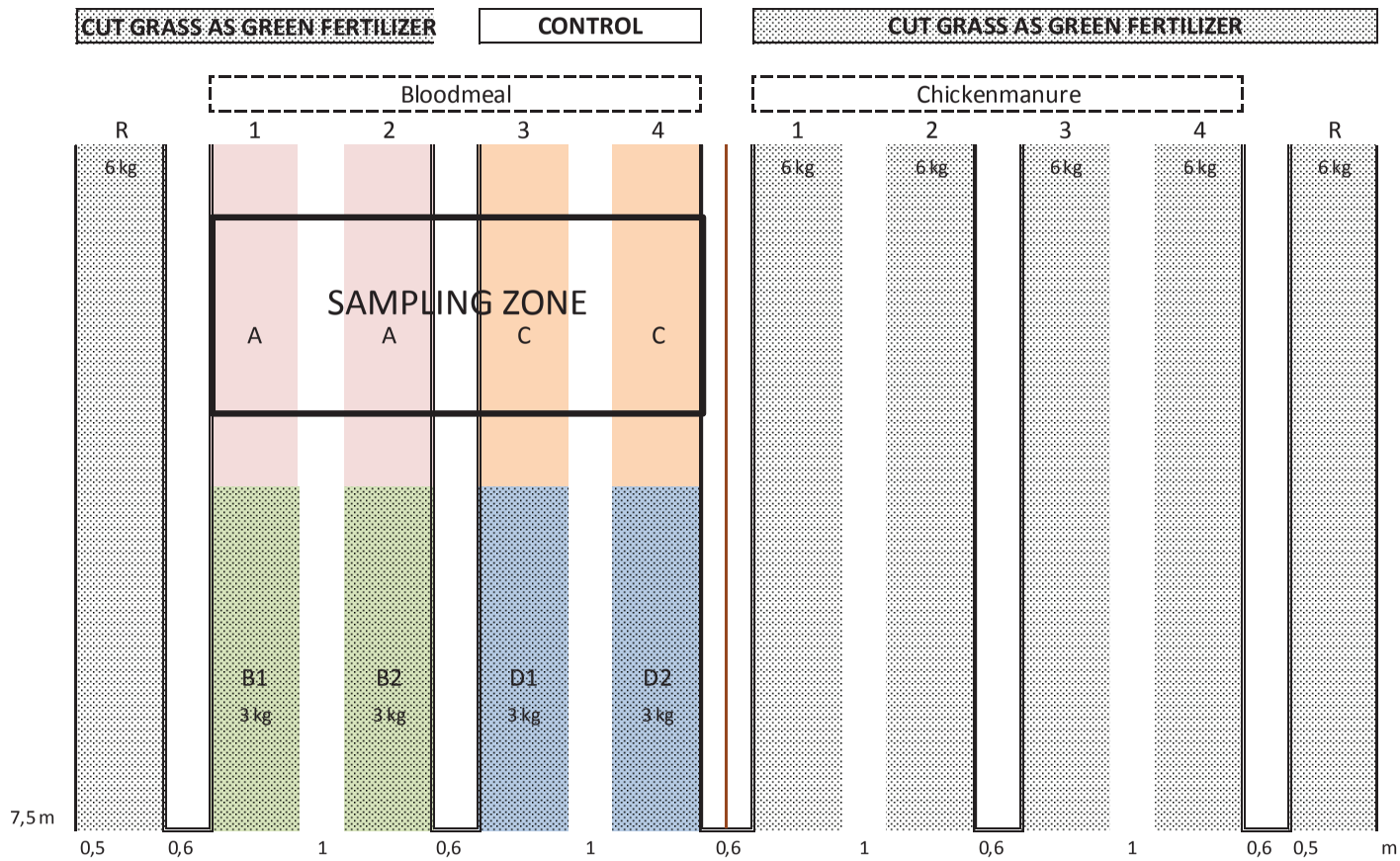
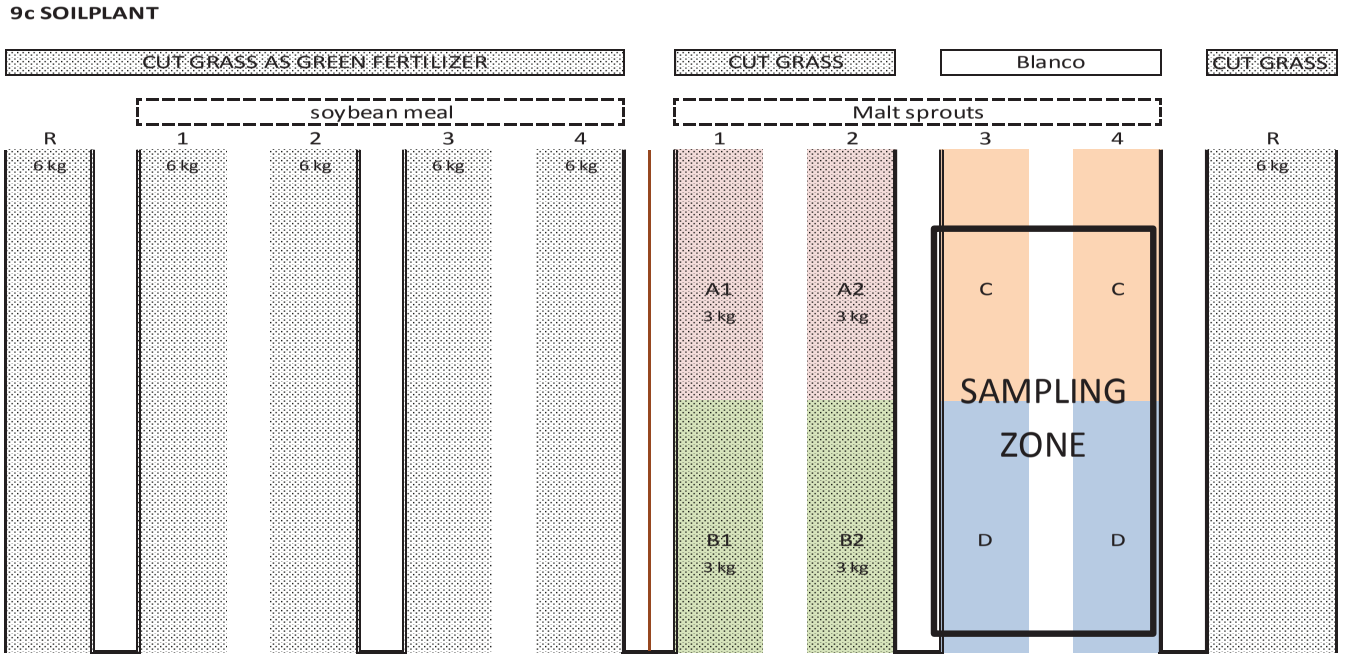
Supplementary information

9a SOILLESS CULTURE

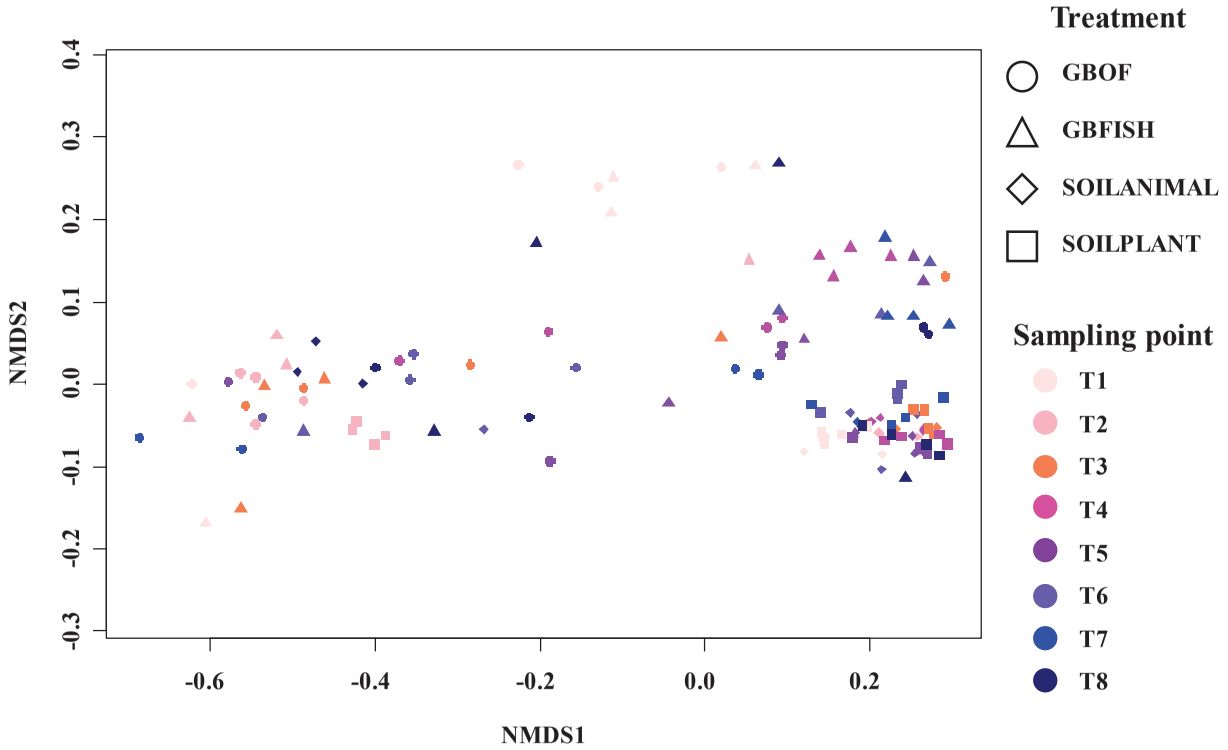


9b SOILANIMAL

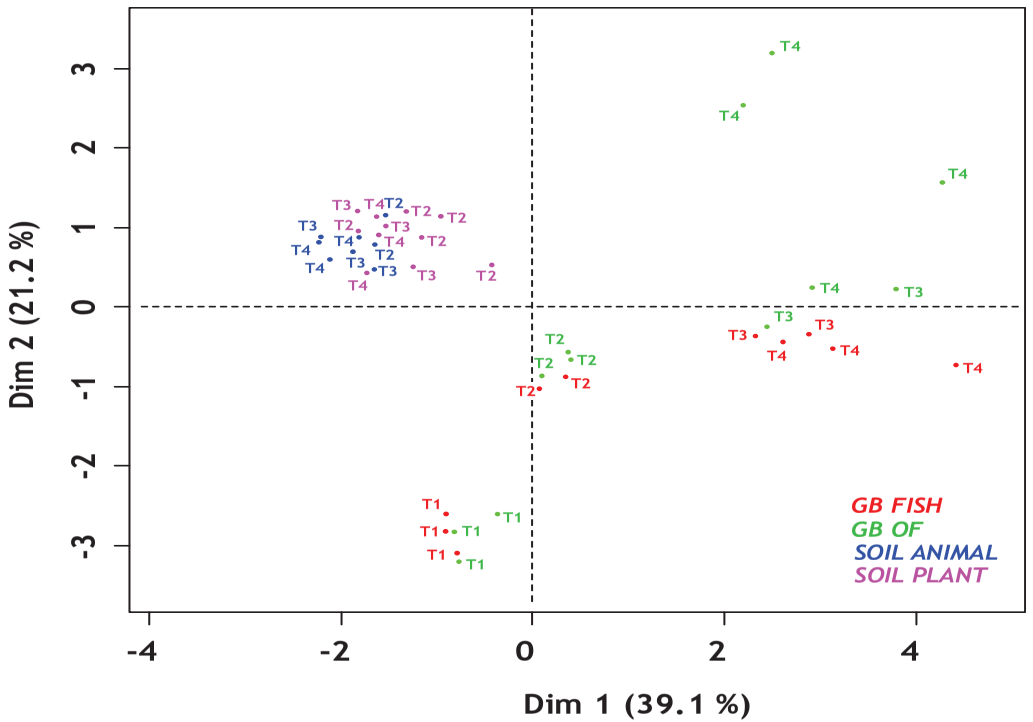




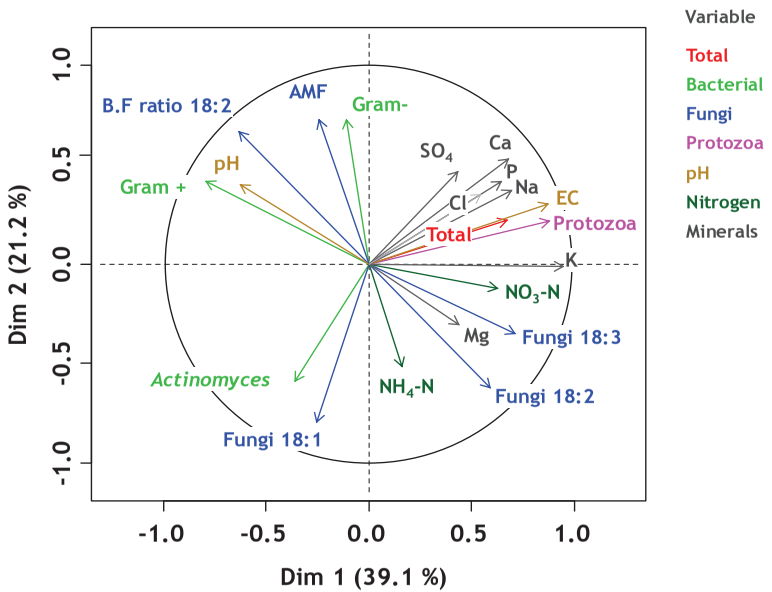
Supplementary figure 6.1: Overview of the experimental set-up. The glass house was divided into three compartments (S91 = 9a GBOF and GBFISH, S92=9b soilanimal and S93 = 9c soilplant) with a surface of 80 m². S91 was subdivided into two part, i.e. red color = GBOF and blue color = GBFISH; S92 was organic soil with animal (blood meal) derived material and S92 was the organic soil with plant-derived material (malt sprouts). In S92 and S93 the previous cultures were tomato in 2014, pepper in 2013 and cucumber in 2012. R= outer rows. Plant density was the same for all the treatment 2.65 plants m^{-2}. Samples were taken in the sampling zone with 40 plants per sampling zone.



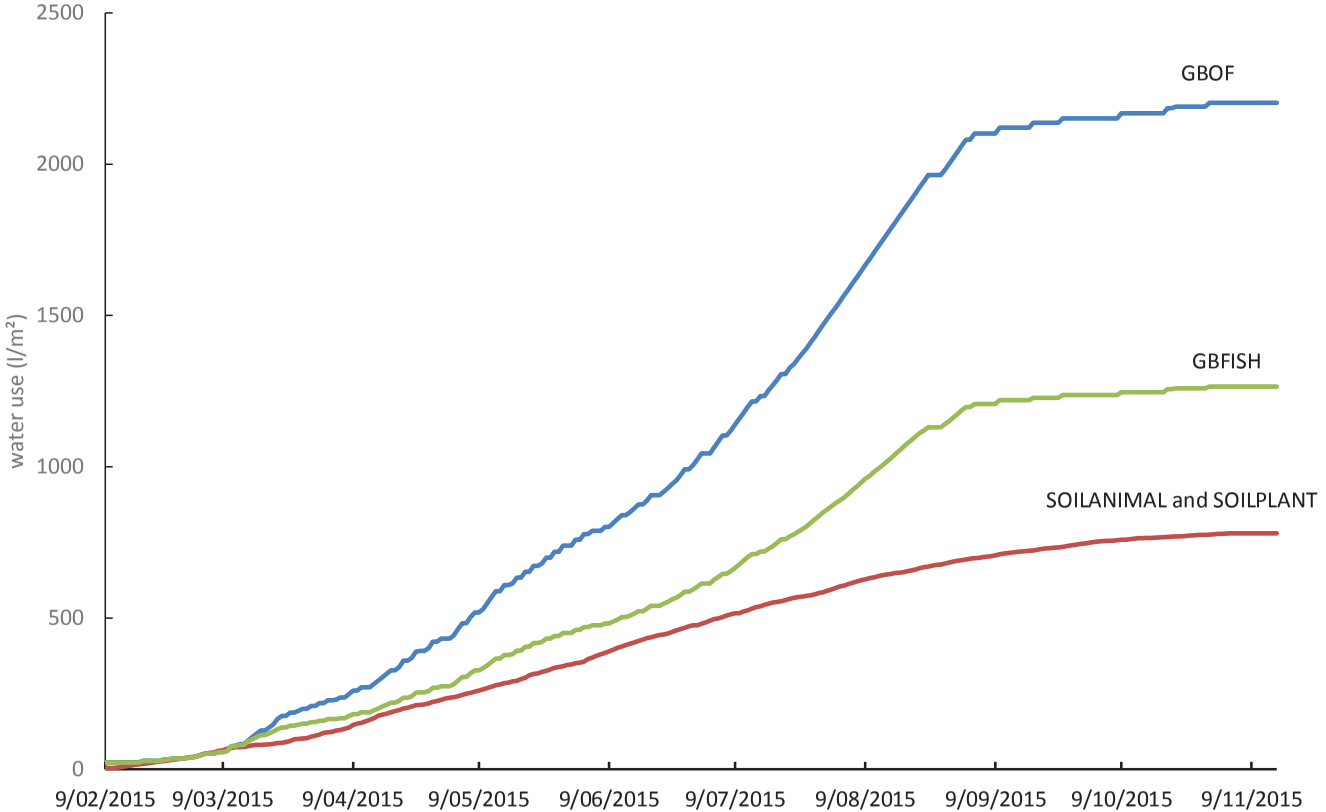
Supplementary figure 6.2: Community structure was significantly different as a result of treatment. Analysis of multivariate homogeneity of group dispersions (or variances) was performed and non-metric multidimensional scaling analysis was used to assess the similarity among bacterial communities in four different soil environments (soil or growing medium) supplied with different carbon fertilizers. Squares indicate communities from soil supplied with plant-derived fertilizer, circles are for growing medium with organic fertilizer, triangles are indicating the communities of growing



Supplementary figure 6.3: Multiple factor analysis in which eleven chemical soil and growing medium characteristics (pH(H_2O), EC, nitrate, ammonium, phosphorus, potassium, calcium, magnesium, sulphate, sodium and chloride) and eight microbial characteristics (gram-positive, gram-negative bacteria, fungi 18:1, fungi 18:2, fungi 18:3, Actinomycetes, arbuscular mycorrhizal fungi and protozoa) were used to discriminate between the different tomato cultivation systems. GBOF= organic growing medium with organic fertilizers, GBFISH= organic growing medium with fish effluent, SOILANIMAL= organic soil that was fertilizer with animal-derived material and SOILPLANT= soil that was fertilized with plant-derived material. "T1"= treatment at time point 1, "T2"= treatment at time point 2, "T3"= treatment at time point 3, "T4"= treatment at time point 4



Supplementary figure 6.4: Plane of the first two dimensions from a MFA in which eleven chemical soil and growing media characteristics (pH(H_2O), EC, nitrate, ammonium, phosphorus, potassium, calcium, magnesium, sulphate, sodium and chloride) and eight microbial characteristics (gram-positive, gram-negative bacteria, fungi 18:1, 18:2, fungi 18:3, Actinomycetes, arbuscular mycorrhizal fungi and protozoa) were used to discriminate between the different tomato cultivation systems. Potassium, protozoa, EC, fungi 18:3, sodium, total bacteria (gram-positive and gram-negative and Actinomycetes), calcium, phosphorus, nitrate, fungi 18:2, chloride, magnesium and sulphate are positively correlated with dimension 1 and Actinomycetes, pH(H_2O), bacteria:fungi 18:2 ratio and the gram-positive bacteria are negatively correlated with Dimension 1. Arbuscular mycorrhizal fungi, gram-negative bacteria, Bacteria fungi 18:2 ratio, calcium, sulphate, phosphorus, gram-positive bacteria, pH(H_2O), sodium and chloride are positively correlated with Dimension 2 and fungi 18:3, ammonium, Actinomycetes, fungi 18:2 and fungi 18:1 are negatively correlated with Dimension 2.



Supplementary figure 6.5: Overview of the cumulative water dosage (L per m²) for the different tomato cultivating systems.

Supplementary table 6.1: Overview of the chemical composition of soilplant and soilanimal before the start of the experiment (8/1/2015) and throughout the whole experimental period. n=1. As bulk density 1.25 t ha^{-1} (Vlaamse zandstreek; Arthur et al. (2011)) was chosen for the 0.1 top soil layer.

Treatment	Date analyze	NO_3-N kg ha^{-1}	NH_4-N kg ha^{-1}	(N-mineral)
SOILANIMAL	8/01/2015	243	< 4	247
SOILPLANT	8/01/2015	196	< 4	200
SOILANIMAL	3/2015	68	<4	72
SOILPLANT	3/2015	97	9	106
SOILANIMAL	5/2015	25	<4	29
SOILPLANT	5/2015	6	7	13
SOILANIMAL	6/2015	18	<4	22
SOILPLANT	6/2015	42	8	50
SOILANIMAL	27/7/2015	16	7	23
SOILPLANT	27/7/2015	17	18	35
SOILANIMAL	4/11/2015	10	<4	14
SOILPLANT	4/11/2015	9	<4	13

Supplementary table 6.2: Overview of the chemical composition of the different fertilizers used. "-" means that the elements was not analyzed or specified. "" means according to the specifications of the supplier. "**" means that the chemical composition was actually analyzed.*

Fertilizer	Composition (%)								
	N_{total}	NO_3^--N	NH_4^+-N	P_2O_5	K_2O	MgO	CaO	Cl	SO_3
Blood meal*	14	-	-	0	0	-	-	-	-
Patentkali*	-	-	-	-	30	10	-	-	42
Magnesium sulphate*	-	-	-	-	-	16	-	-	32
Malt sprouts*	3	-	-	0	0	-	-	-	-
Antys MgS**	0	-	0	0	0	9	0	0	18
Biosyr**	9.35	-	0.53	3.96	9.08	0.11	3.98	0.68	10.9
Nutrikali**	2.4	-	0.1	0.3	4.9	0.1	0.1	1.1	1.1
SP**	0	-	0	0.001	58.6	0	0	0	0
Calcium chloride 33 %*	0	-	0	0	0	0	16.5	21	0
Calsal vlb 51%*	8.7		-	-	-	-	8.7	-	-
Amnitra vlb 51%*	18	9	9	-	-	-	-	-	-
Potassium nitrate*	13	13	-	-	45	-	-	-	-
Magnesium sulphate*	-	-	-	-	-	16	-	-	32
Monopotassium phosphate*	-	-	-	52	34	-	-	-	-
Potassium sulphate *	-	-	-	-	50	-	-	-	45

Supplementary table 6.3: Overview of the fertilizers used and the total amount used for the different treatments. GBOF = soilless culture system with organic growing medium and organic fertilizer, GBFISH = soilless culture system with organic growing medium and fish, SOILANIMAL= organic soil with animal-derived material as fertilizer and SOILPLANT= organic soil with plant-derived material as fertilizer

Treatment	Type of fertilizer used	Total amount of fertilizer used ($g\ m^{-2}$)
GBFISH	Calsal vlb 51% ($Ca(NO_3)_2$)	1855
	Amnitra vlb 51% (NH_4NO_3)	557
	Potassium nitrate (KNO_3)	959
	Magnesium sulphate ($MgSO_4$)	279
	Monopotassium phosphate (KH_2PO_4)	368
	Potassium sulphate (K_2SO_4)	288
GBOF	Antys MgS	827
	Biosyr	1292
	Nutrikali	1870
	SP	404
	$CaCl_2$	921
	Libremix	49
SOILANIMAL	Blood meal	180
	Patentkali	163
SOILPLANT	Malt sprouts	1001
	Patentkali	163

Supplementary Table 6.4: Overview of the chemical composition of the four different organic fertilizers (Nutrikali, ANTYS MgS, Biosyr and SP). "-": means that this element was not determined in the fertilizer

	Nutrikali	**ANTYS MgS**	**Biosyr**	**SP**
Total Nitrogen (%)	2.35 ± 0.1 2	-	9.35 ± 0.23	-
Organic nitrogen (%)	2.25 ± 0.11	-	8.81 ± 0.22	-
NH_4^+-N (%)	0.056 ± 0.003	-	0.532 ± 0.027	-
NO_3^--N (%)	0.042 ± 0.002	-	0.012 ± 0.001	-
P_2O_5 (%)	0.257 ± 0.013	-	3.96 ± 0.20	0.0007 ± 0.0001
K_2O (%)	4.93 ± 0.25	-	9.08 ± 0.23	58.6 ± 1.5
CaO (%)	60.113 ± 0.006	-	3.98 ± 0.20	-
MgO (%)	0.121 ± 0.006	0.725 ± 0.036	0.110 ± 0.005	-
SO_3^{2-} (%)	1.11 ± 0.06	1.28 ± 0.06	10.9 ± 0.3	-
Na_2O (%)	1.97 ± 0.10	-	0.836 ± 0.042	-
Cl (%)	0.839 ± 0.042	-	0.676 ± 0.034	-
Organic matter (%)	0.839 ± 0.042	-	57.4 ± 1.4	-

Supplementary table 6.5: Correlations between microbial community composition and chemical characteristics in four different tomato cultivating systems across time points, indicated by the Multiple Factor Analysis. Dimensions of the MFA can be described by the categorical variables included in the analysis. For each categorical variable (growing medium and time point), a one-way analysis of variance was performed with the coordinates of the samples on the axis, explained by the time point or growing medium type. Then, for each level of the category (i.e. time point 1, time point 2 or time point 3 or growing medium GB), a Hotelling T^2-test was used to compare the average of the category with the general average (using the constraint P i αi = 0, αi = 0). For instance, the coordinates of the relative abundance of family "x" at GB at time point 1 were compared with the average coordinates of the relative abundance of family "x" in GB. The P value associated to this test is transformed to a normal quantile to assess whether the mean of the category is significantly less or greater than 0. Negative values indicate negative correlations.

Dimension	Variance	Descriptor	Estimate (R^2)	P value	Taxon and or chemical characteristics	Correlation	*P* value
					K	0.9505387	<0.0001
					Protozoa	0.882805	<0.0001
					EC	0.8713766	<0.0001
		GBOF	1.5492	0.00261	Fungi.18.3	0.7156968	1E-07
		GBFISH	1.451668	0.015256	Na	0.7015117	3E-07
					Total	0.6796157	0.000001
					Ca	0.6779943	1.1E-06
					P	0.6499159	4.3E-06
DIM 1	39.1%				NO_3^-N	0.6348602	8.3E-06
					Fungi.18.2	0.5960548	3.92E-05
					Cl	0.548466	0.000205
					Mg	0.4452108	0.003536
					SO_4^{2-}	0.4374311	0.004237
					Actinomycetes.	-0.3618954	0.020071
					pH(H2O)	-0.6273887	1.13E-05
					Bacteria Fungi18.2 ratio.	-0.6355404	8.00E-06
					Gram-positive bacteria	-0.7987867	<0.0001
					AMF	0.729531	1E-07
DIM 2	21.2%				Gram-negative bacteria	0.7288208	1E-07
					Bacteria Fungi ratio.18.2.	0.670518	1.6E-06

	tpt4	1.178248	0.014011	Ca	0.5255753	0.000418
	SOILPLANT	0.8708145	0.016697	SO_4^{2-}	0.4707461	0.001895
	SOILANIMAL	0.7376762	0.103984	P	0.4189329	0.006407
				Gram-positive bacteria	0.4164783	0.006757
				pH(H2O)	0.4021037	0.009157
				Na	0.3751969	0.015642
				Cl	0.3520146	0.024004
				Fungi.18.3	-0.3528578	0.023645
				NH_4^+-N	-0.517281	5.34E-04
				Actinomycetes	-0.5900243	4.91E-05
				Fungi.18.2	-0.6236232	1.33E-05
				Fungi.18.1	-0.8010289	<0.0001
DIM 3				NH_4^+-N	0.675832	1.2E-06
				Total	0.6403078	6.5E-06
	GBOF	1.27019	5.96E-05	Cl	0.5425307	0.000248
				pH(H2O)	0.4863632	0.001263
				Mg	0.3706436	0.017054
				P	0.3569491	0.021966
				Fungi.18.2	-0.446345	0.003443
				Fungi.18.3	-0.49035	1.14E-03
				NO_3^-N	-0.722229	1.00E-07
				Actinomycetes	0.5783302	7.49E-05
DIM 4	SOILANIMAL	-0.6524775	0.005355	Gram-negative bacteria	0.5103223	0.000653
				SO_4^{2-}	0.4863936	0.001262
				NH_4^+-N	0.4423815	0.003778
	tpt3	0.4160831	0.051796	Mg	0.3565815	0.022113
				Gram-negative bacteria	0.3443766	0.027467
DIM 5				Cl	-0.313677	0.045812
				Na	-0.320351	0.041154

	Fungi.18.1	-0.347512	0.025998
	P	-0.39069	0.011552

CHAPTER 7: PLANTS RATHER THAN FERTILIZER DRIVE RHIZOSPHERE BACTERIAL COMMUNITY IN ORGANIC GROWING MEDIUM BLENDED WITH RECOVERED NUTRIENTS

This chapter is in preparation:

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In preparation

Abstract

Intensive agriculture and horticulture depend on fertilizer input to sustain food production. However, synthetic fertilizer production is associated with a high carbon footprint and pollution. Recovered nutrients are promising alternatives that may contribute to a sustainable crop production in combination with organic growing medium. These recovered nutrients can replace fertilizers commonly used in growing media. We hypothesized that both plant host may affect the microbial community in the root and bulk zone and recovered nutrients applied, such as ammonium struvite and organic fertilizer. We investigated the microbial community in soilless culture systems in combination with two plant, two different fertilizer over time, in terms of microbial community structure and functionality, i.e. ammonia oxidation rate. We showed that the community composition of the growing medium in combination with a plant and no plant was distinctly different and this could be attributed to differences in chemical factors in soilless culture systems. The use of organic fertilizer in combination with organic growing media result in an even and diverse microbial community, however species richness was not affected. Inorganic fertilizer, such as struvite results in microbial communities with a lower evenness and diversity. The rhizosphere microbial community composition was influenced by plant development stage and by the amount and type of fertilizer used in soilless culture systems and finally no differences in AOB abundance between the rhizosphere compared to the bulk zone were found challenging the theory that microorganisms are better competitors for nitrogen than plants.

1. Introduction

Intensive agriculture and horticulture heavily rely on the input of inorganic and organic fertilizers to sustain food production (Erisman et al. 2008). European farmers and consumers spend around €15.5 billion per year on synthetic fertilizers, with 76% of this value for nitrogen (N), 16% for phosphorus (P), and 8% for potassium (K). For optimal plant growth, however, timing, ratio and quantity are fundamental, because the nutrient demand of the plant may not be concomitant with the nutrient release from the fertilizers (Oertli 1980; Prasad et al. 2001). Inorganic fertilizers have in common that the nitrogen is present as ammonium and/or nitrate and they are popular as they are rapidly plant available given their high solubility (Sonneveld and Voogt 2009b). Organic fertilizers, on the contrary, have in common that the majority of the nitrogen and partly also phosphorus is present in organic form and is released gradually through microbial conversion. Organic fertilizers can be produced on-farm such as slurries, poultry manures, digestate or off farm coming from food industry residues (Hajdu et al. 2015). In addition, P and N can be removed and recovered from wastewaters, sludge or even through crystallization with magnesium, yielding struvite ($MgNH_4PO_4.6H_2O$), a slow releasing fertilizer (De-Bashan and Bashan 2004; El Diwani et al. 2007).

The organic nitrogen mineralization relies directly on the microbial nitrogen conversion and results in a release of ammonia. The release of ammonia depends on the mineralization rate of the organic nitrogen. Ammonia oxidizing bacteria archaea and bacteria (AOA and AOB) are found in the vast majority of terrestrial ecosystems (Thion et al. 2016) and oxidize ammonia (NH_3) to nitrite (NO_2^-). Nitrite is subsequently oxidized to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB). Consequently, the presence of nitrate depends on both the presence of ammonium and the ammonia and nitrite oxidation rates (Boudsocq et al. 2012). Depending on the pH of the growing medium ammonia withdraws an H^+ ion to form ammonium resulting in an increase of the pH (Hornung 2005; White 2013). Struvite can be used as fertilizers, however, nutrients, such as ammonium, are released at a slower rate compared with other inorganic fertilizers. The solubility of struvite depends on the ionic strength, pH and temperature. The solubility of struvite determined in deionized water was found to be 9.6 mg N L^{-1} or 21.3 mg P L^{-1} at 25°C. Predicting the solubilisation rate of struvite is complex, as it is controlled by a combination of factors such as thermodynamics of liquid-solid equilibrium, phenomena of mass transfer between solid and liquid phases, kinetics of reactions, and several physico-chemical parameters (Bhuiyan et al. 2008). However, the solubilisation of struvite occurs only, when the activities of magnesium, ammonium and phosphate are below the thermodynamic solubility product (Ksp) of struvite. The release of ammonium from struvite may also be impacted by microorganisms due to the assimilation of ammonia by the ammonia oxidizing bacteria.

Plants, such as tomatoes and lupine, can take up ammonium, nitrate and organic nitrogen. Tomato plants are known be sensitive to ammonium resulting in chlorosis of the leaves and showing a stunted growth (Britto and Kronzucker 2002). In addition, the narrow leafed lupine is capable of fixing atmospheric nitrogen, and is also able to assimilate ammonium and nitrate (Unkovich et al. 1994). Mentioned research showed that narrow leafed lupine (*Lupinus angustifolius* L.) showed the highest yields in combination with nitrate and the lowest yield in combination with ammonium (Barłóg and Grzebisz 2000). The uptake of ammonium by plants is from an energetic point of view less costly than the uptake and assimilation of nitrate (Boudsocq et al. 2012). However, ammonium uptake can cause severe toxicity symptoms. Plants also have high demands for potassium, calcium and magnesium and increased ammonium uptake is negatively correlated with the uptake of mentioned cations.

Consequently, the uptake of nitrate is interesting to fulfil the high demand for other essential nutrients. Nitrogen is present in different forms in organic growing media blended with organic fertilizers and struvite, however little is known about the plants' preference for ammonium or nitrate under these conditions. Plants take up most of mineral nutrients through the rhizosphere, where microorganisms interact with root exudates. Rhizosphere was described for the first time by Hiltner (1904). According to Berendsen et al. (2012) the rhizosphere is the narrow zone of soil that is influenced by root secretions, however little is known about these effects in soilless culture systems in combination with organic growing media. Rhizosphere effects, according to (Kandeler et al. (2002); Mendes et al. 2014; Teixeira et al. 2010; Turner et al. 2013), were determined by analyzing the soil closely adhering to roots. It is obvious that plants and microorganisms compete for the nitrogen present in the growing medium. Research indicates that competition might be stronger on ammonium than on nitrate (Hodge et al. 2000), and if microorganisms are the better competitors for ammonium, this might lead to an increased production of nitrate by the nitrifiers. This suggests that nitrification and its control by plants may play a central role in the outcome of competition for nitrate and ammonium between two species (Lata et al. 2004). There is abundant evidence that plants use N that is left over from microbial metabolism, at least in non-agricultural soils (Hodge et al. 2000). This suggests, that microorganisms efficiently compete for ammonium with the plants. Management practices, such as nitrogen fertilization, influence plant traits, such as leaf area, fresh and dry weight. Consequently, the effects of fertilization on the microbial community may also be indirectly mediated through plant trait changes (Legay et al. 2016). Indeed, plants impact growing media through the exudation of organic substances in the rhizosphere, which influences microbial community structure and activity, nutrient cycling, pH and finally plant growth (Bardgett et al. 2014). As indicated by Thion et al. (2016), some studies suggest that AOA, rather than AOB, are favored in the rhizosphere (Chen et al. 2008; Herrmann et al. 2012), while others indicate the opposite (Glaser et al. 2010; Wei et al. 2011). The most important factors determining this niche specialization between AOA and AOB are believed to be the pH of the growing medium, with AOA generally predominant in an acidic environment (Nicol et al. 2008) and the ammonia concentration. AOA have a greater affinity for ammonia and exhibit greater sensitivity to inhibition by high ammonia concentration (Prosser and Nicol 2012). As a result of this,0 AOB are often found to dominate in soil and growing media with greater inorganic N availability (Verhamme et al. 2011). Several contradictory reports in the literature indicate plant or soil type as major factors influencing the microbial community (Berg and Smalla 2009; Girvan et al. 2003; Grayston et al. 1998; Nunan et al. 2005). The effect of plants on the rhizosphere and bulk zone microbial community, and on the ammonia oxidizing community, has often been studied in natural soils with complex plant ecosystems, however little is known about these interaction in soilless culture systems.

While progressive progress is made to understand the microbial community in soils, a large knowledge gap exists concerning soilless culture microbial communities in combination with organic growing media. Moreover, their structure and sensitivity towards the use of inorganic and organic fertilizers and the type of plant. The aim of this study was to determine the effect of recovered nutrients (struvite and one organic fertilizer) blended with organic growing medium on the plant development, functionality, i.e. ammonia oxidation rate and composition of the microbial community associated with the bulk zone and the rhizosphere over time. We used plants with different nitrogen uptake strategies: tomato, known for its high root exudation capacity (Heddes 2012) and capable of taking up organic nitrogen, ammonium and nitrate and lupine, which has nitrogen-fixing ability, as model plants. Agricultural practices, such as fertilization, may impact plant microbiome composition. This work

addresses four questions concerning the microbial community composition in soilless culture systems: (1) Is the bulk zone microbial community structure of a growing medium without a plant distinctly different in comparison to a growing medium with a plant, i.e. tomato or lupine? (2) Do plants drive rhizosphere microbial community composition in organic growing media blended with recovered nutrients? (3) Is the rhizosphere microbial community more even, diverse and distinctly different from the bulk zone microbial community? (4) Do plants affect abundance and activity of rhizospheric microbial communities in combination with tomato and fertilizer used?

2. Material and Methods

2.1 Experimental setting and growing medium and recovered nutrients used

Fertilizers (struvite and the organic fertilizer) were mixed with the organic growing medium (GB) at a dose of 100 mg N L^{-1} growing medium. The struvite was recovered from a wastewater treatment plant (The Laboratory of Chemical and Environmental Engineering, Lequia, University of Girona, Spain) and the organic fertilizer (8 % w/w organic-N, 2.18 % w/w P and 4.97% w/w K) was a commercially available fertilizer (Frayssinet, France). The chemical composition of the recovered nutrients can be found in Table 7.1.

Table 7.1: Chemical composition of the recovered nutrients. ND= not determined. stdev=standard deviation

Parameters	***Organic fertilizer*** *(mean±stdev)*	***Struvite ($NH_4MgPO_4.6H_2O$)*** *(mean±stdev)*
Total N (%)	*7.78 ± 0.19*	*ND*
Organic-N (%)	*6.89 ± 0.17*	*ND*
NH_4-N (%)	*0.36 ± 0.02*	*6.6±0.2*
NO_3-N (%)	*0.017 ± 0.001*	*ND*
Urea-N (%)	*0.51 ± 0.03*	*ND*
P in mineral acid (%)	*2.19 ± 0.06*	*13.2± 0.4*
K in water (%)	*4.93 ± 0.12*	*ND*
Ca total (%)	*5.70 ± 0.14*	*ND*
Mg total (%)	*0.57 ± 0.03*	*10.6± 0.2*
S total (%)	*2.14 ± 0.05*	*ND*
Na total (%)	*0.48 ± 0.02*	*ND*
Organic matter (%)	*54.4 ± 1.4*	*ND*

The organic growing medium (GB, Grow Bag, Peltracom, Belgium) consisted of a mixture of white peat (H2-H4 on the von Post scale (Von Post 1926) [40% v/v], Irish peat [40% v/v] and coconut fiber [20% v/v]). The average fresh bulk density (n=4) of the growing medium was 225.04 kg/m³, determined according to EN12580. The growing medium had a gravimetric water content of 0.50±0.02 kg kg^{-1}.

Plants (Tomato and lupine) were grown in climate chambers at the Institute Plant Sciences (IBG-2; Forschungszentrum Jülich GmbH, Jülich, Germany) under the following controlled conditions: day

length of 16 h, day/night temperatures of ~24/18°C and illumination was <400 µmol m^{-2} s^{-1} between 06:00 and 22:00 hours local time. Rhizotrons with dimensions of 60 cm x 30 cm x 2 cm were filled with organic growing medium.

Each rhizotron (Figure 7.1) was filled with 1.1 kg of the growing medium, equivalent to 5 L of growing medium per rhizotron. Part of the growing medium was sterilized using gamma–irradiation (BGS, Wiehl, Germany) at minimal doses of 50 kGy to eliminate the native microbial community associated at the start of the experiment, and was used as a control. The use of gamma irradiation as a method for soil or growing medium sterilization for laboratory experiments has been recommended over other sterilization techniques (McNamara et al. 2003).

The rhizotrons, consisting of black polyethylene, had one removable side of transparent polycarbonate plate so that planar optodes (Presense GmbH, Regensburg, Germany) could be installed. One optode was placed on the glass with special glue (GE Bayer Silicone, Leverkusen, Germany) at 27 cm from the top and the second optode was placed at a depth of 16.5 cm measured from the first optode or 43 cm from the top. The optodes had a sensitive side directed to the growing medium, whereas the glue side was directed to the glass. To place the optodes, the glass was removed carefully from the rhizotron, keeping it at horizontal position. After placing the optodes, it was screwed back onto the rhizotron.

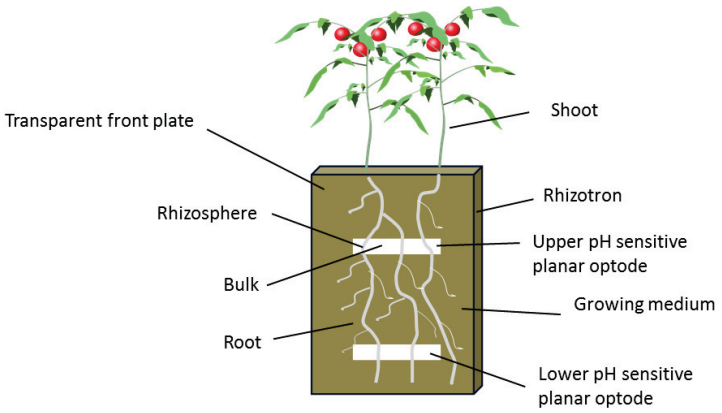


Figure 7.1: Overview of the experimental design

2.2 Tomato and lupine as model plants

Tomato (*Solanum lycopersicum* L. x *Solanum habrochaites* Maxifort, Monsanto Vegetable Seeds, Bergschenhoek, The Netherlands) and lupine (*Lupinus angustifolius*) seeds were germinated on filter paper and were transplanted at 2 days old (2 seedlings of tomatoes per rhizotron and 1 seedling for the lupine). The seeds were planted at a depth of 2 cm and in contact with the Plexiglas. Rhizotrons were maintained at an angle of 45° during the growing period to ensure the maximum number of visible roots growing along the glass. All plants were supplied with demineralized water and each rhizotron received 100 mL of 1/3 Hoagland nutrient solution at the beginning of the experiment, and

60 mL deionized water 3 times per week to maintain a growing medium water content of ~30% (volumetric water content).

2.3 Sampling of the bulk zone and the rhizosphere

Root growth was followed over time, and the visible root length at the surface of the rhizotron represented approximately 30% of the total root system length, consistent with previously reported data (Hurd 1964; Nagel et al. 2012). Time point 1 was considered as the time when the rhizotrons were filled and seeds were placed on top of the rhizotron. When the roots reached the center of the first optode about 20 days after sowing, 50% of all the rhizotrons were opened and growing medium samples were collected and considered the first harvest (time point 2). Bulk zone (± 0.5 g) and rhizosphere samples (± 0.2 g) were collected in the zone of the upper pH sensitive optode, for microbial community analyses. Table 7.2 gives an overview of the samples taken for the chemical, microbial analysis and the activity test at the different time points.

Table 7.2: Overview of the time point in relation to the samples taken for analysis. DAS= days after sowing

	Samples for chemical analysis	Samples for microbial analysis	Samples for activity test (tomato)
Time point 1 (0 DAS)	X	X	
Time point 2 (20 DAS)	X	X	
Time point 3 (34 DAS)	X	x	X

Optodes were used for guided sampling to determine optimal sampling times and locations with pH changes in the bulk zone and the rhizosphere. Two weeks after the first harvest, the remaining 50% of the rhizotrons were opened and measurements of shoot and roots were performed (time point 3). The experiment ended at this point because the plant roots reached the bottom of the rhizotrons. Leaf area, shoot fresh and dry weight and chemical composition of the growing medium was measured at harvest. In total, 203 samples were collected for determination of the microbial community composition, of which 194 samples were selected for sequencing analysis. Only 194 samples were used for Illumina sequencing, because samples that did not pass the quality check, were not further used. Indeed, once nucleic acid extraction was done, the presence, quantity and/or quality of the extract prior to engaging in downstream applications was verified. The presence of nucleic acids was assessed and this was achieved by running them on an agarose gel and subsequently staining the gel with a fluorescent dye. Visualization was performed with a gel dock containing a UV transilluminator enabling excitation of the dye. In addition, samples for the ex situ tests were taken at the 3rd time point for the tomato experiment only.

2.4 Physicochemical analysis of the growing medium

The physicochemical characteristics of the organic growing medium were determined at the start and during the growing period. Chemical analysis (pH(H2O), electrical conductivity, ammonium, nitrate, phosphorus, potassium, calcium, magnesium, sulphate, sodium, and chloride) were performed as described by Gabriels et al. (1998b). Potassium, phosphorus, calcium, magnesium, iron and manganese

were extracted (1:5 vol/vol) in ammonium acetate and measured with ICP. The electrical conductivity (EC), pH(H2O), ammonium (NH_4^+), nitrate (NO_3^-), sulphate (SO_4^{2-}) and sodium (Na) were measured in a 1:5 v/v water extract following EN 13038, EN 13037 and EN 13652, respectively. Nitrate was measured with an IC ion chromatograph. Ammonium was measured by steam distillation.

2.5 Sampling procedure for microbial community analysis

Time point 1 was considered as the time when the rhizotrons were filled and seeds were placed on top of the rhizotron. Time point 2 was considered, when the root tips of at least 50% of the plants reached or passed the center of the upper optode. Time point 3 was considered, when the root tips of the remaining plants reached or passed the center of the lower optode. At each time point (2 and 3) the rhizotrons were removed one after another from the growth chamber. Pictures of the entire rhizotron and the roots and plants were taken to visualize the total root surface. These pictures were used to estimate the total root length (primary and secondary) of the tomato and lupine. Moreover, pictures of the 2 optodes were taken with a special device (see section 2.6) to visualize pH changes. The Plexiglas plates with the attached optodes were carefully removed from the rhizotron without destroying the roots. A copy of the Plexiglas plate was placed again on the rhizotron leaving the space open where the optodes were initially located. The sampling zone for the microbial community analysis equaled the surface of the optodes. Afterwards samples from the rhizosphere and the bulk zone were taken with sterilized (70% ethanol by volume) material (tweezer and scalpel). Within this sampling zone roots were cut with a sterilized scalpel and these roots were used to quantify the rhizosphere, i.e. the weight of the fresh growing medium attached to the roots. The growing medium, attached to the roots was sampled, with an approximate distance < 1 mm from the roots and a weight equal to the weight as previously determined. This sample was considered as a rhizosphere sample. Bulk zone sample was considered as the growing medium not attached the roots and with a distance of more than > 10 mm from the roots. The weight of these samples was approximately 0.5 g. The fresh weight of each sample was determined and these samples were immediately stored at 80°C for community analysis.

2.6 Detection of pH dynamics in the rhizosphere with planar optodes

Planar optodes, used for guided sampling and pH measurement in the rhizosphere, are sensor foils with embedded fluorescent molecules, which emit a characteristic pattern of fluorescence after excitation depending on the analyte concentration (Gansert and Blossfeld (2008); Holst and Grunwald (2001); and Blossfeld et al. (2013). A camera that is sensitive to the emission range of the optode detects this fluorescence signal, which serves as information carrier. Further, using light as an information carrier allows for separation of the sensor (the planar optode) and detector (the camera). In this experiment, the planar optodes (Figure 7.1) were used for non-invasive *in situ* measurement of pH dynamics in the rhizosphere and the bulk zone. The used planar optodes had a measuring threshold between pH(H_2O) = 5.5 and 8.30.

2.7 Ex situ tests for the potential ammonia oxidation activity tests

High-throughput batch activity tests were adapted from (Courtens et al. 2016) and performed to determine the potential ammonia oxidation rate in the bulk and rhizosphere from the organic growing medium without fertilizer, with organic fertilizer and struvite, respectively. *Ex situ* potential ammonia oxidation rates were measured after the second harvest (33 days after sowing or third time point) in the tomato experiment. Four samples were collected of each treatment (no fertilizer, organic and

struvite and rhizosphere) from the rhizotrons, in the bulk zone and near the first installed planar optode in the rhizosphere. As an internal control, samples were collected from gamma-sterilized growing medium. The bulk and the rhizosphere samples (0.132 ± 0.078 g) were stored for 48 h at 21°C before the batch activity tests were started. Samples were subsequently mixed with a P buffer to a final ratio of 30 mg of growing medium per mL of buffer and vortexed for 1 min at maximum speed. The buffer solution (pH 6.5) further contained final concentrations of 0.774 g P L^{-1} (KH_2PO_4/K_2HPO_4), 0.1 g $NaHCO_3$ L^{-1} and 25 mg N L^{-1} as $(NH_4)_2SO_4$. Then, six replicates of 260µL each were transferred to 96-well plates and incubated in a MB100-4A Thermo shaker (Hangzhou Allsheng Instruments, China) at room temperature, at 600 rpm and sealed with parafilm (Benis NA, Neenah, WI, USA) to minimize evaporative losses. Ammonium concentrations were determined using a Tecan infinite M200 PROplate reader (Männedorf, Switzerland), following the Berthelot reaction (Bucur et al. 2006).

2.8 DNA extraction

Total DNA was extracted from the growing medium samples using the Power Soil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), following the manufacturer's instructions. Five hundred milligrams were used from the bulk and 0.1 g from the rhizosphere. Concentration and quality of DNA were measured based on the absorbance at 260 and 280 nm in a Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.9 Abundance of total bacteria, Archaea, NOB (*Nitrobacter* and *Nitrospira*), AOB and AOA in the bulk zone and rhizosphere

Quantitative PCR assays of 49 samples from bulk and rhizosphere from tomato plants were completed using an ABI StepOnePlus real-time PCR system. Reactions were performed in a total volume of 20 µl, with 10 µl of 2x iTaq universal SYBR Green supermix (Bio-Rad Laboratories, Hercules, CA, USA), 1 of µl DNA template (50 ng μL^{-1}), 1 of µl of each primer (Supplementary table 7.1 and Supplementary table 7.2) and nuclease-free water volume adapted according to the primer concentration used. Amplifications were run as follows: initial denaturation for 2 min at 95°C, followed by 40 cycles of 15 s denaturation at 95°C, 30 s annealing at a specific annealing temperature (Supplementary table 7.1 and Supplementary table 7.2) and 30 s extension at 60°C. At the end of the qPCR run, a melting curve analysis was performed to confirm product specificity (60-95°C, ΔT per 15 s = 0.3°C). Quantification was performed using a standard curve based on known concentrations of DNA standard dilutions from 10^7 copies μl^{-1} to 10^2 copies μL^{-1}. All qPCR analyses were conducted in triplicate.

2.10 Illumina library generation

High-throughput amplicon sequencing of the V3 – V4 hypervariable region (Klindworth et al. 2012) was performed with the Illumina MiSeq platform according to the manufacturer's guidelines at LGC Genomics GmbH (Berlin, Germany). Contigs were created by merging paired-end reads based on the Phred quality score (of both reads) heuristic as described by Kozich et al. (2013) (Kozich et al. 2013) in Mothur (Schloss et al. 2009) (v.1.33.3). Contigs were aligned to the SILVA database and filtered from those with (i) ambiguous bases, (ii) more than 8 homopolymers, and (iii) those not corresponding to the V3 – V4 region, which resulted in a removal of 75 % of the sequences. The sequencing errors were removed using IPED, a recently released algorithm dedicated to denoise MiSeq amplicon sequencing data (available at http://science.sckcen.be/en/Institutes/EHS/MCB/MIC/Bioinformatics/IPED). Chimera removal was performed using the CATCh tool (Mysara et al. 2015) set in de novo mode, which

resulted in removal of an additional 16% of the sequences. Sequences were classified using the RDP trainset (Cole et al., 2007) version 9, removing those with Eukaryota, Mitochondria or Chloroplast classification. The sequences were clustered into operational taxonomic units (OTUs) at 97% identity level with UPARSE (Edgar 2013) on default settings (v7.0.1001_i86linux32) via the sortbysize, cluster_otus, and usearch_global commands. Quality of the sequencing and post-processing pipeline was verified by incorporating mock samples (n = 12 species) in triplicate into the same sequencing run. A total of 868,162 of reads were obtained. After examining read counts, if any OTU was not classified up to genus level, the consensus sequence was blasted using the NCBI database to obtain the taxonomic classification. Singletons that remained unclassified were culled. Richness, Fisher's diversity, Shannon, Simpson and inverse Simpson indices were calculated to assess alpha diversity within each sample. Pielou's index was used as indicator of evenness in the community. Differences in alpha diversity and evenness measures among treatments were compared using a repeated measures mixed model in SAS (version 9.4, SAS Institute, Cary, USA), with fertilizer (no fertilizer, organic fertilizer and struvite), plant (no plant, lupine, and tomato), location (bulk versus rhizosphere) as a fixed effect for the third time point. Hence, the differences in the diversity measures could be attributed to plant, fertilizer and location or to the interaction of the three factors. Beta diversity estimates based on Chao and Bray-Curtis indices were used to examine dissimilarity and determine the impact of experimental factors on microbial community structure. nMDS was employed to visualize the differences among samples, using the vegan package in R (Oksanen et al. 2007). Stratified permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was conducted to indicate the significance of each covariate (time, fertilizer and growing medium pre-treatment) on the microbial community of the bulk and rhizosphere. ANOVA was applied to reveal whether the distribution of the genera was different between plants (Oksanen et al. 2007). Because of the over-dispersion in the OTU data, a zero-inflated count model was used to assess the effect of fertilizer and plant and the interactions between plant*fertilizer on each individual genus, in both rhizosphere and bulk growing medium. Zero-inflated models explain the excess of zeros by modeling the data as a mixture of a Poisson distribution or a negative binomial distribution. When a zero count is observed, there is the zero-inflation probability, because the observation came from the always-zero distribution. When the underlying count distribution is a Poisson distribution, the model is called a zero-inflated Poisson distribution and if the count distribution is a negative binomial distribution, the mixture is called a zero-inflated negative binomial distribution. The final model was selected based on the Akaike Information Criterion (AIC). Differences among library size sample were accounted for with the offset option in proc GLIMMIX in SAS (Paschold et al. 2012). P values for each comparison were converted to q-values that were then used to identify differences in relative abundances of bacterial genera while controlling false discovery rate (FDR) at the 5% level (Storey 2015).

2.11 Multivariate statistics

Differences in physicochemical characteristics among growing medium supplemented with different fertilizers were compared using a mixed model in SAS (5 version 9.4, SAS Institute, Cary, USA). Pearson correlations (Supplementary Table 7.9, Supplementary Table 7.10 and Supplementary Table 7.11) were used to determine the interactions between the physicochemical characteristics and significance was assumed at $P < 0.05$. Eleven variables were included in the analysis (pH(H_2O), conductivity, nitrate-N, ammonium-N, phosphorus, potassium, calcium, magnesium, sulphate, sodium, chloride). Multiple Factor Analysis (MFA) assisted to detect how the relative abundances of bacterial genera differed in growing media harboring either of the two plants. The function MFA from the FactoMineR package (Lê

et al. 2008) was performed in R. Parametric bootstrapping was applied to construct confidence ellipses around the barycenter of the samples included on each covariate (time/fertilizer/plant), and thus visualize whether the bacterial abundances were significantly different among any of these categorical descriptors. If the ellipses were not overlapping, the bacterial abundances were significantly different; incomplete overlap indicated that bacterial abundances were significantly different in the samples outside the ellipse (Dehlholm et al. 2012). Statistical differences in ammonia oxidation rate were analyzed using a longitudinal mixed model in SAS. A random slope model was used with time point, fertilizer and location (bulk or rhizosphere) as fixed factors and all interactions were considered. Technical replicates (n=6) were nested within each biological replicate (n=4). Unstructured covariance structure was used, assuming that the variance differed between the rhizosphere and the bulk zone.

3. Results

3.1 Recovered nutrients impacted plant growth

Plant ($P < 0.05$), fertilizer ($P < 0.05$) and time point ($P < 0.05$) had a significant effect on the leaf area, fresh weight and dry weight (Table 7.3).

Table 7.3: Influence of fertilizer type (no fertilizer -NOF, organic fertilizer – ORG and ammonium struvite-STR) on the growth performance of tomato and lupine plants in a non-sterile organic growing medium in function of time. n= 5. Tpt 1= time point 1; tpt2 = time point 2 (=harvest 1) and tpt 3= time point 3 (harvest 2). NA = not available, (P <0.05) SEM= standard error of the mean.

Variable	Plant	Tpt	Fertilizer			SEM	P value			
			NOF	ORG	STR		Plant	Fertilizer	Time point	Plant*Fertilizer* Time point
Leaf area (cm^2)	Lupine	1	NA	NA	NA	46.0	<0.001	<0.001	<0.001	<0.001
		2	23.9	25.7	22.4					
		3	52.9	86.9	65.6					
	Tomato	1	NA	NA	NA					
		2	6.64	182.3	102.1					
		3	95.3	990.3	734.9					
Fresh weight (g)	Lupine	1	NA	NA	NA	1.83	<0.001	<0.001	<0.001	<0.001
		2	1.11	1.25	1.24					
		3	2.58	4.50	3.36					
	Tomato	1	NA	NA	NA					
		2	0.14	3.92	2.16					
		3	2.46	35.18	25.22					
Dry weight (g)	Lupine	1	NA	NA	NA	0.191	<0.001	<0.001	<0.001	<0.001
		2	0.139	0.145	0.143					
		3	0.408	0.607	0.473					
	Tomato	1	NA	NA	NA					
		2	0.01	0.208	0.16					
		3	0.24	3.08	2.24					

The largest mean leaf area (321.3 cm²) was found when organic fertilizer was provided. Mean total leaf area decreased by 28% and 86% when struvite or no fertilizer were used, respectively. Organic fertilizer application yielded the largest total leaf area, and highest fresh and dry weight (on either plant), whereas struvite resulted in a decrease of the mean total leaf area, and of fresh and dry weight (Table 7.3).

3.2 Recovered nutrients affect pH and N-dynamics of the growing medium of each plant over time

The pH(H_2O) of the bulk growing medium measured in a 1:5 v/v water extract was significantly influenced by plant, fertilizer and time point ($P < 0.05$, Supplementary Table 7.3). The overall pH(H_2O) was 5.6 ± 0.03 at the start, increased to 6.2 ± 0.03 at the second time point and decreased again to 5.7 ± 0.03 at the third time point, in all plants. Organic fertilizer and struvite resulted in similar pH(H_2O) changes in the growing medium.

Ammonium concentration increased continuously in the struvite treatment (Supplementary table 7.3), whereas it only reached a peak at time point 2 when organic fertilizer was provided. Phosphorous and magnesium were also consistently increased in the struvite treatment but not in the organic fertilizer (Supplementary table 7.3). Nitrate concentration was significantly influenced by plant ($P<0.01$), fertilizer ($P < 0.05$) and time point ($P < 0.05$), showing an inconsistent trend when organic fertilizer was supplied to tomato plants. In contrast, nitrate concentration continuously increased over time in lupine with organic fertilizer. Moreover, nitrate also continuously increased over time when struvite was supplied on either plant (Supplementary table 7.3). We confirmed that plant, fertilizer and time had an influence on the nutrient dynamics in the bulk zone.

3.3 Detection of pH dynamics in the rhizosphere with planar optodes

The pH monitoring in the rhizosphere and the bulk zone via the optodes revealed that the investigated tomato plants modified their rhizosphere pH. Distinctive pH patterns were only found in combination with the organic fertilizer and not in combination with struvite or the no fertilizer treatment. It is very likely that pH changes were not visualized, because these pH changes were not continuously monitored. We observed in the pictures (Figure 7.2 and Figure 7.3) and after calibration of these values an increase of the pH(H_2O) in the rhizosphere when the root is crossing the optode.

The rhizospheric pH changed from 6.2 to 7.6. This effect is decreased over time, meaning that the pH(H_2O) in the rhizosphere is decreasing again to pH(H_2O) 6.9 at the harvest point (33 days after sowing). The pH(H_2O) of the bulk zone increased slightly to a value of 6.2, that matches also with the real value measured in the growing medium with a pH(H_2O) meter.

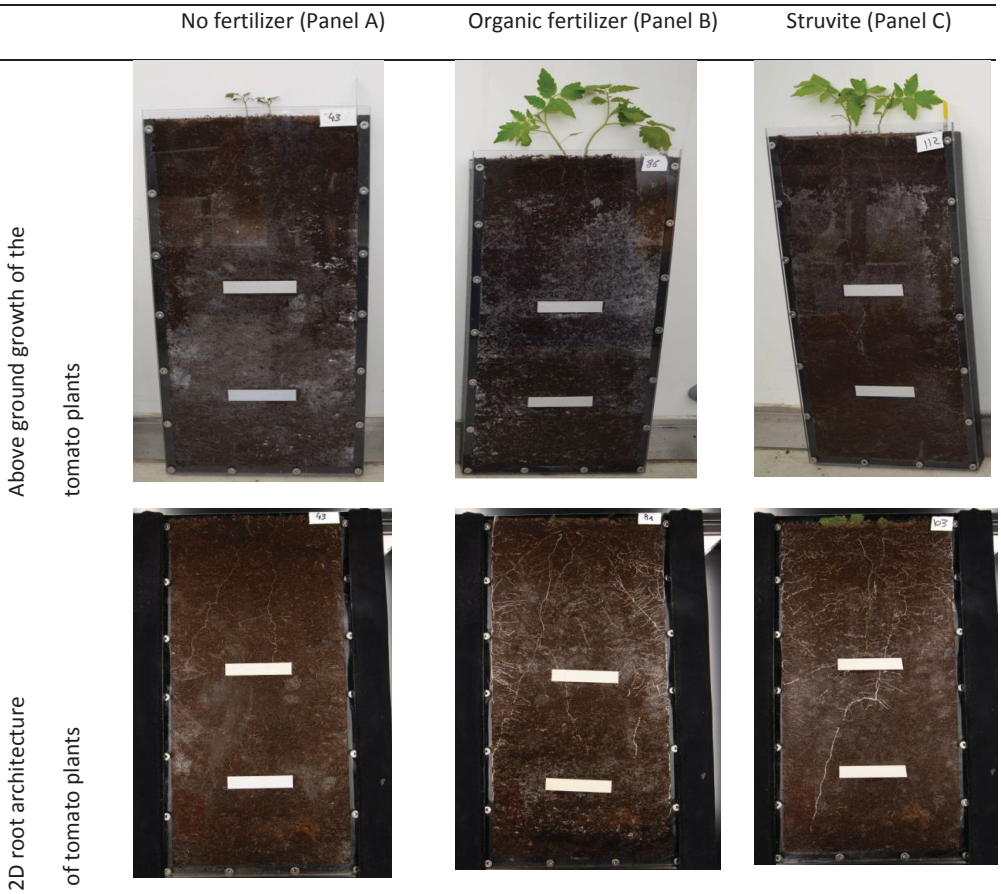
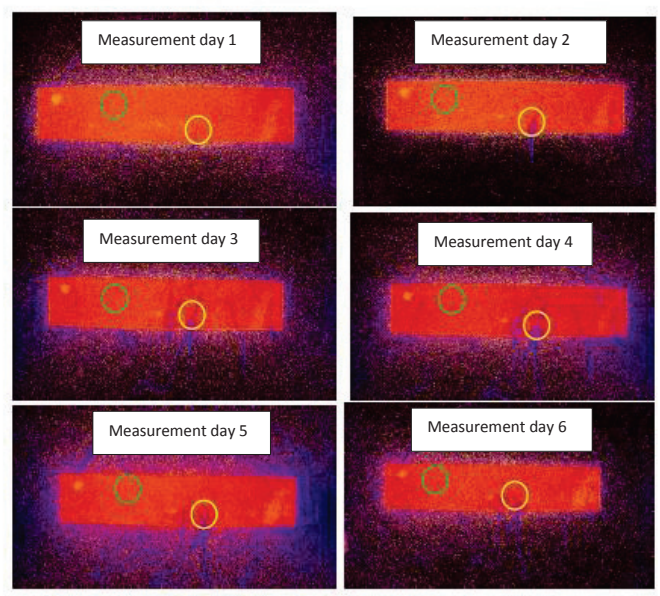


Figure 7.2: Picture of the experimental setup as seen through the transparent window of the rhizotrons with the optodes installed. Panels (A, B, C) were shot at the time of destructive harvesting which corresponded to the second harvest, time point 3) and we can clearly see the roots crossing the planar optodes in combination with the organic fertilizer.



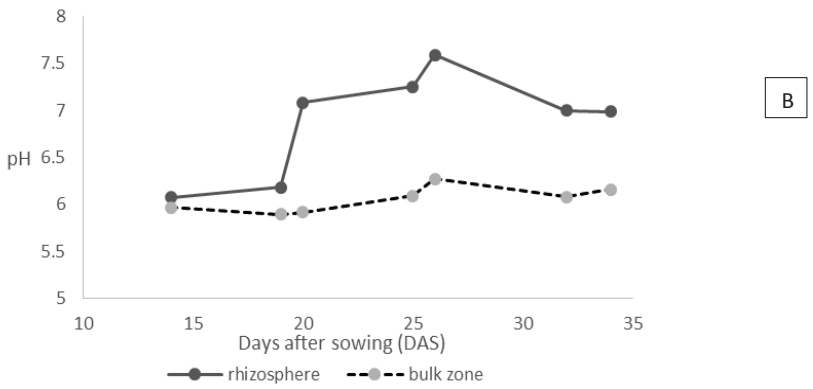


Figure 7.3: from left, to right and top to bottom (A), sequence of pH change in the rhizosphere of the tomato plants measured with the upper pH optodes under the organic fertilizer treatment. Green circles show, where the pH values were determined for the "bulk zone", and yellow circles show, where the pH values were determined for the rhizosphere. (B) Evolution of the pH changes in the rhizosphere and the bulk zone in function of time (days after sowing 8/5/2015).

3.4 Effect of recovered nutrients on the abundance of total bacteria, total Archaea, ammonia oxidizing bacteria, *Nitrobacter* and *Nitrospira* in the bulk zone and rhizosphere

The total amount of bacterial *amoA* gene copy number per g of growing medium from tomato plants was significantly influenced by fertilizer treatment ($P < 0.05$, Table 7.3) and was not significantly influenced by location at the second harvest (Table 7.3).

Table 7.4: Influence of fertilizer type (no fertilizer -NOF, organic fertilizer – ORG and ammonium struvite-STR) and the location (rhizosphere versus bulk) on the total and the relative amount of copy numbers per g of growing medium in non-sterile organic growing media with tomato. n= 8. NS = no significant effect. Numbers are µ±σ; LOD= limit of detection was 10^3 for amoA bacteria, 16S Bacteria, 16S Archaea, 16S Nitrobacter and 16S Nitrospira and 10^2 for the archaeal amoA gene copy number. LOD = limit of detection

	Fertilizer			Location		Effect (P-value)		
	NF	**ORG**	**STR**	**Bulk**	**Rhizosphere**	**Fertilizer**	**Location**	**Fertilizer *Location**
16S Bacteria	$1.0x10^8$± $4.8x10^7$	$2.0x10^8$± $4.8x10^7$	$2.5x10^8$± $4.6x10^7$	$1.3x10^8$± $4.6x10^7$	$2.4x10^8$± $3.9x10^7$	NS	0.04	NS
16S Archaea	$1.6x10^7$± $7.2x10^6$	$2.9x10^7$± $7.2x10^6$	$3.5x10^7$± $6.9x10^6$	$1.7x10^7$± $5.8x10^6$	$3.5x10^7$± $5.8x10^6$	NS	0.0002	NS
Nitrobacter	$1.7x10^6$± $8.7x10^5$	$3.2x10^6$± $8.7x10^5$	$3.3x10^6$± $8.4x10^5$	$1.4x10^6$± $7.0x10^5$	$4.1x10^6$± $7.0x10^5$	NS	0.008	NS
Nitrospira	$3.7x10^4$± $3.1x10^4$	$1.2x10^5$± $2.8x10^4$	$1.3x10^5$± $3.1x10^4$	$5.6x10^4$± $2.2x10^4$	$1.4x10^4$± $2.5x10^4$	NS	0.02	NS
amoA Bacteria	<LOD[a]	$5.1x10^6$± $1.0x10^{6b}$	$1.4x10^6$± $1.7x10^{6b}$	$2.3x10^6$± $1.6x10^6$	$4.2x10^6$± $1.3x10^6$	0.05	NS	NS
Nitrobacter / 16S bacteria	2.3±0.7%	1.4±0.7%	2.7±0.7%	1.8±0.6%	2.7±0.6%	NS	NS	NS
Nitrospira/ 16S bacteria	0.04±0.01 %	0.05±0.01 %	0.05±0.01 %	0.05±0.01 %	0.05±0.01%	NS	NS	NS
amoA Bacteria/ 16SBacteria	<LOD[a]	2.6±0.3%[b]	0.7±0.8%[a]	1.3±0.7%	2.0±0.5%	0.03	NS	NS
16S Nitrobacter/ 16S Nitrospira	29.2±7.8%	32.3±7.0%	24.5±7.1%	25.5±5.6%	31.8±6.3%	NS	NS	NS

The lowest *amoA* copy number was recorded when no fertilizer was supplied; no differences were found between struvite and organic fertilizer (Table 7.3). The location (bulk zone or rhizosphere) had a significant effect on total bacteria, total Archaea, *Nitrobacter* and *Nitrospira* ($P < 0.05$, Table 7.3, Supplementary table 7.4). Total bacteria were higher in the rhizosphere compared to the bulk zone, except for *Nitrospira*, where the opposite was observed (Supplementary table 7.4).

The relative AOB abundance (copy number ratio of bacterial *amoA*: total bacteria) was significantly impacted by fertilizer type ($P < 0.05$), but not by location or interaction of the factors. AOB was 100 times higher in the organic fertilizer in comparison with the other treatments (Supplementary table 7.4). The archaeal *amoA* gene copy number was below the detection limit of $2.5x10^3$ gene copy numbers per µL (Supplementary table 7.4).

The relative NOB abundance (ratio of the *Nitrobacter+Nitrospira*:total bacteria) was not significantly influenced by fertilizer used and location (Table 7.4). *Nitrobacter* are higher in absolute and relative numbers in comparison to *Nitrospira* within the NOB community in the organic growing medium. No significant shifts in the relative *Nitrobacter/Nitrospira* ratio associated with location, fertilizer treatment or the interaction of both were detected (Table 7.4).

3.5 Plants influence the microbial community composition in the rhizosphere

Multiple Factor Analysis (MFA) of the bulk zone showed that *Nitrosospira,* magnesium, phosphorous and the pH(H_2O) were positively correlated with lupine in the not sterile bulk zone, supplied with struvite at time point 2 (Supplementary table 7.5). They were represented in Dimension 1 ($P < 0.0001$), accounting for 13.5% of the variance in relative abundances among all the samples. *Frankiaceae,* ammonium, phosphorus, electrical conductivity, sodium and chloride were the main variables correlated with struvite at the third time point and tended to be associated only with tomato plants. Dimension 2 ($P < 0.0001$) explained these variables, which accounted for 10.96% of the variance (Supplementary table 7.5). Dimension 3 described the community in the tomato, supplied with organic fertilizer at time point 2, which was positively associated with *Opitutus,* sulphate, sodium, chloride and potassium (Supplementary table 7.5) and accounted for 8.02%. Dimension 4 was associated with the community and chemical characteristics influenced by the absence of plant in sterile growing medium supplied with struvite, at the second time point and accounted for 7.63% of the variance among samples. No covariates were correlated with dimension 5 (Supplementary table 7.5).

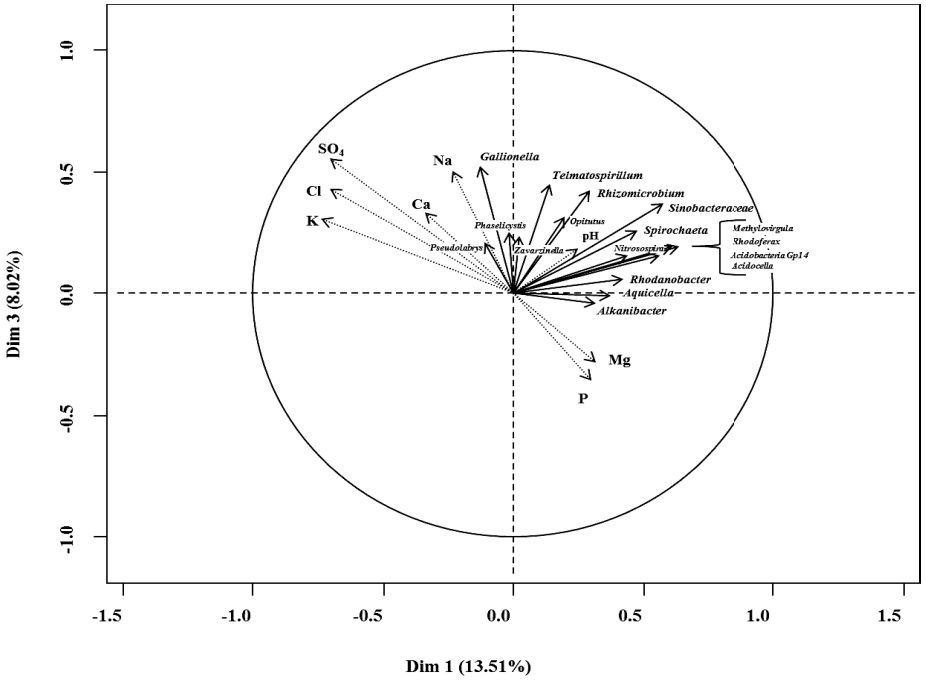


Figure 7.4: Correlation circle of variables on the first and third dimensions of the Multiple Factor Analysis (MFA) performed on physicochemical characteristics and microbial community of bulk growing medium supplemented with different fertilizers and harboring two different plant species. The first dimension of the MFA described the growing medium supplemented with struvite and harboring lupine, while the third dimension was constructed by the variables associated with the growing medium supplemented with organic fertilizer and harboring tomato plants. As a result, these two dimensions were projected in the map

The growing medium supplemented with struvite and harboring lupine (Dim 1) was positively correlated with *Alkanibacter*, *Aquicella*, *Rhodanobacter*, *Acidoccella*, *Nitrosospira*, *Spirochaeta*, *Acidobacteria Gp14*, *Rhodoferax*, *Methylovirgula* and *Sinobacteriaceae*, while the growing medium supplemented with organic fertilizer and harboring tomato plants (Dim 3) was positively correlated with physicochemical characteristics of the growing medium like potassium, sulphate, calcium, sodium and chloride (Figure 7.4).

Confidence ellipses (confidence interval CI=95%) revealed that the impact of plant, time and sterilization on the microbial community in the bulk zone was greater than that of fertilizer (Figure 7.5), confirming the dissimilarity of the relative abundances between growing medium harboring different plants.

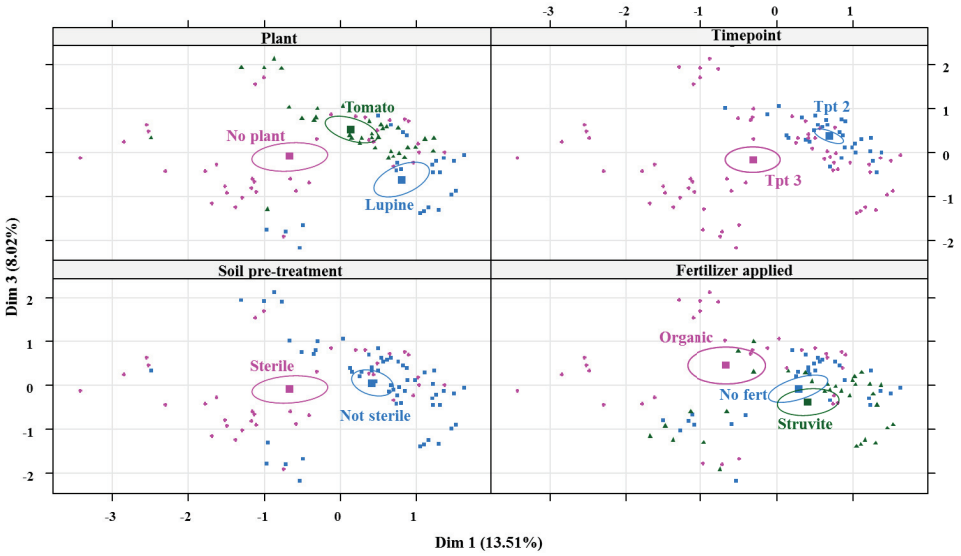


Figure 7.5: Microbial community shifts of pre-treated (sterile versus non-sterile) bulk zone harboring two different plants, supplemented with fertilizer and followed over time. Multiple Factor Analysis revealed variations in the relative bacterial abundances and ellipses show confidence Intervals (CI) of 95% for each sample type. The first dimension of the MFA described the growing medium supplemented with struvite and harboring lupine, while the third dimension was constructed by the variables associated with the growing medium supplemented with organic fertilizer and harboring tomato plants. As a result, these two dimensions were projected in the map

MFA was independently performed for the relative abundances detected in the rhizosphere. This analysis uncovered that the rhizosphere of lupine plants supplied with struvite, at time point 2 was described in the Dimension 1 of the MFA, accounting for 13% of the variance in relative abundance among all samples. The genera associated with tomato at the second time point were represented in Dimension 2 ($P < 0.0001$, Supplementary Table 7.6), accounting for 12% of the variance. Dimension 3 was associated with the rhizosphere community influenced by the absence of fertilizer treatment (Supplementary Table 7.6), accounting for 5% of the variance among all samples. Dimensions 4 and 5 described the community in the lupine at time point 2 (Supplementary Table 7.6). Overlapping CI suggested that the influence of fertilizer in the rhizosphere was not significant (Figure 7.7).

MFA (Figure 7.6) showed microbial community shifts of the bulk growing media and the rhizosphere of tomato at tpt 2 (dimension 2) and lupine at tpt 2 (dimension 3). The overall microbial communities of lupine and tomato are independent and they are not impacted by the use of the fertilizer. MFA shows that the plant effect is determinant on the differences in the relative abundances of the communities in the different growing media microbiomes (Figure 7.6).

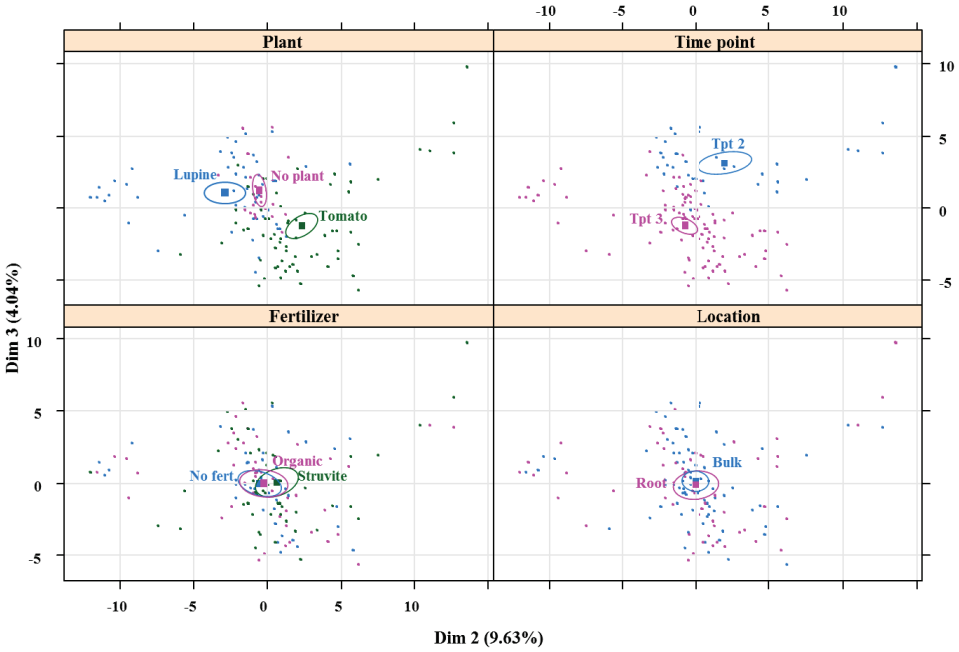


Figure 7.6: Microbial community shifts of the bulk growing media and the rhizosphere of tomato at tpt 2 (dimension 2) and lupine at tpt 2 (dimension 3). The overall microbial communities of lupine and tomato are independent and they are not impacted by the use of the fertilizer. MFA shows that the plant effect is determinant on the differences in the relative abundances of the communities in the different growing media microbiomes.

In addition, MFA was also used to indicate significant differences in the rhizosphere in relative bacterial abundances as a result of plant, time and fertilizer. The second dimension of the MFA (Figure 7.7) described the growing medium harboring tomato plants, while the fourth dimension was constructed by the relative abundances of the bacteria associated with the growing medium harboring lupine. The confidence ellipse of the rhizosphere shows significant separation of the microbial community by plant and time (Figure 7.7). This indicates that the bacterial abundances in the rhizosphere were significantly different regardless of fertilizer supplementation. Figure 7.7 shows that the community structure in rhizosphere was significantly different because of plant host ($P < 0.05$).

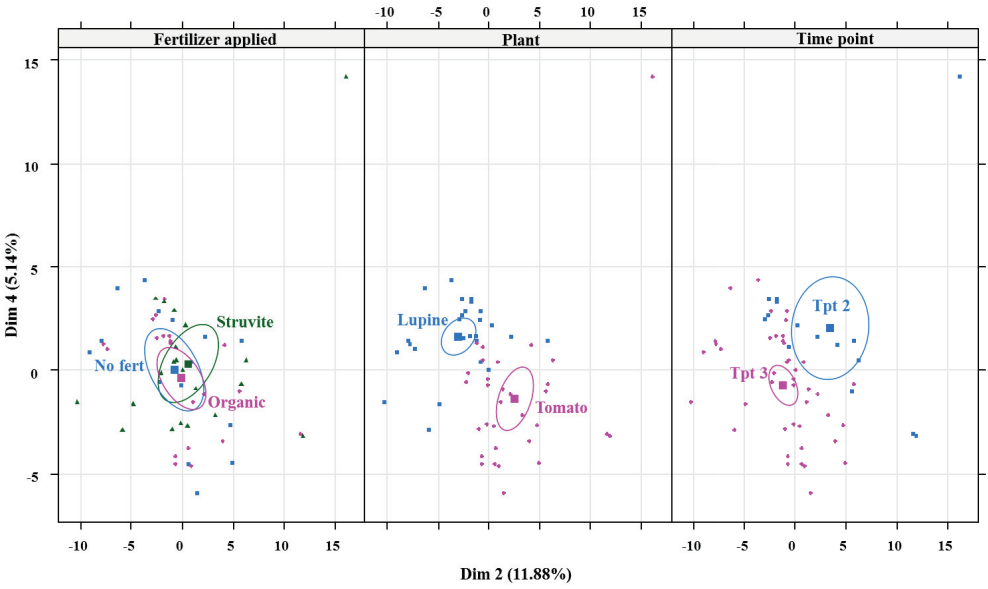


Figure 7.7: Microbial community shifts of rhizosphere in growing media harboring two different plants, supplemented with fertilizer and followed over time. Multiple Factor Analysis revealed variations in the relative bacterial abundances and ellipses show confidence Intervals (CI) of 95% for each sample type. The second dimension of the MFA described the growing medium harboring tomato plants, while the fourth dimension was constructed by the relative abundances of the bacteria associated with the growing medium harboring lupine. As a result, these two dimensions were projected in the map and variations in the bacterial relative abundances over time and in response to plant and fertilizer were detected.

PERMANOVA was performed to indicate the significance of each covariate (time, fertilizer and growing medium pre-treatment) on the microbial community of the bulk. Our analysis confirmed that plant and time point significantly contributed to the differences in the relative abundances of the bacterial genera ($P < 0.05$). Evenness, diversity and total species were higher when no fertilizer was supplied, followed by the organic fertilizer treatment and then by struvite ($P < 0.05$, Table 7.5). Moreover, these metrics increased over time ($P < 0.05$). Differences in the relative abundances of the bacterial genera suggested the presence of distinctive microbial communities associated with the rhizosphere, which differed between plants.

3.6 Alpha diversity measures are influenced by fertilizer, plant type and location

We calculated all the alpha diversity indices, to account for sensitivity differences among indices. Generally, a significant effect of the fertilizer and the plant used on the diversity measures was found. No significant effect was found for the location, i.e. rhizosphere or bulk zone. With respect to species richness tomato showed a significant higher amount (P=0.04) of species than lupine. No effect of fertilizer and location was found on species richness. However, there is a trend that the no fertilizer treatment has a higher amount of species compared to organic fertilizer and struvite and the bulk zone had a higher amount off species compared to the rhizosphere.

Fertilizer was significantly influenced evenness of the microbial community (*P*=0.039), but not by plant and location. The microbial community in combination with the no fertilizer treatment and the treatment with struvite were more even compared to the organic fertilizer treatment. Biodiversity, as indicated by the several biodiversity indices, was significantly influenced by fertilizer (*P*=0.0001) and plant (*P*=0.02). The no fertilizer treatment showed a higher diversity index than the organic fertilizer treatment followed by the struvite. The diversity index was significantly higher in combination with tomato compared to lupine.

Table 7.5: Effect of location (rhizosphere and bulk zone), fertilizer (no fertilizer, organic and struvite) and plant (lupine and tomato) on species richness (total species), diversity (Shannon, Fisher's alpha, Simpson and Inverse Simpson indices), and evenness (Pielou's index) for the third time point (=second harvest) (n=81). Lupine (n=36); tomato (n=45); NOF= no fertilizer (n=25); ORG= organic fertilizer (n=27) and STR= struvite (n=29), rhizosphere (n=40; bulk (n=41). NS = no significant effect.

	Location (mean±standard error)		Fertilizer (mean±standard error)			Plant (mean±standard error)		Effect			
	Rhizosphere	Bulk	NOF	ORG	STR	Lupine	Tomato	Location	Fertilizer	Plant	loc*fertilizer* plant
Total species	274±10	292±9	301±12	286±11	262±11	269±10[a]	297±9[b]	NS	NS	0.04	NS
Pielou	0.5521± 0.0018	0.5488± 0.0018	0.5544± 0.0023[b]	0.5508± 0.0022[a]	0.5462± 0.0022[c]	0.5490± 0.0019	0.5519± 0.0017	NS	0.039	NS	NS
Shannon	3.08± 0.02	3.1± 0.02	3.2± 0.02[a]	3.1± 0.02[b]	3.00± 0.02[c]	3.0± 0.02[a]	3.1± 0.01[b]	NS	<0.0001	0.002	NS
Simpson	0.7456± 0.0002	0.7454± 0.0002	0.7463± 0.0003[a]	0.7457± 0.0003[b]	0.7445± 0.0003[c]	0.7451± 0.002[a]	0.7459± 0.0002[b]	NS	<0.0001	0.02	NS
Fischer Alpha	59.0± 1.6	61.7± 1.6	65.2± 2.1[a]	61.3± 1.9[b]	54.5± 1.9[c]	57.7± 1.7[a]	63.0± 1.5[b]	NS	0.001	0.02	NS
Inverse Simpson	3.931± 0.003	3.929± 0.003	3.942± 0.004[a]	3.933± 0.004[b]	3.914± 0.004[c]	3.924± 0.003[a]	3.935± 0.003[b]	NS	<0.0001	0.02	NS

The alpha diversity indices was not influenced by location, which means that the number of species (richness), the relative abundances of each of these species (evenness) and the pool of species (diversity) is the same in bulk and roots. However, the beta diversity measures were significantly different. This seems to be contradictory. Beta diversity measures indicated that there are differences in species composition between the two locations; however, it is likely that not all the same species are present in both environments. Maybe the number of species is the same, but not the way they are

distributed within time points, within fertilizers and within plants. Moreover, we have a 3-way interaction, so it is difficult to pinpoint that one single factor has a higher impact than the other factors.

4. Discussion

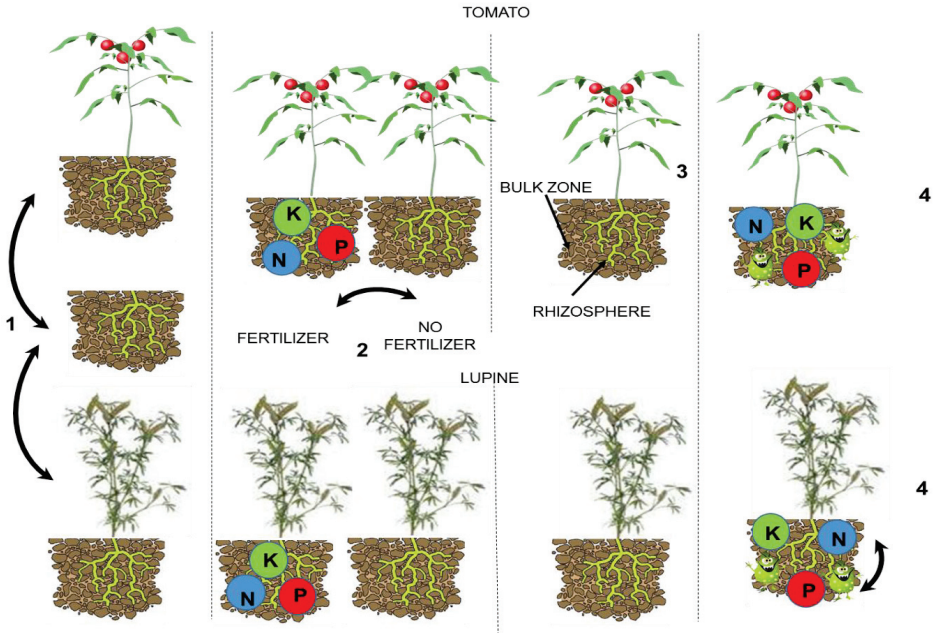


Figure 7.8: Guideline for the discussion. (1) Is the bulk zone microbial community structure of a growing medium without a plant distinctly different in comparison to a growing medium with a plant, i.e. tomato or lupine? (2) Do plants drive rhizosphere microbial community composition in organic growing media blended with recovered nutrients? (3) Is the rhizospheric microbial community more even, diverse and distinctly different from the bulk zone microbial community? (4): Do plants affect abundance and activity of rhizospheric microbial communities in combination with tomato and fertilizer used?

4.1 The bulk zone microbial community structure of a growing medium without a plant is distinctly different compared to a growing medium with a plant, i.e. tomato or lupine

Figure 7.8 gives a general guideline for the discussion. Confidence ellipses (confidence interval CI=95%) revealed that the impact of plant, time and sterilization on the microbial community in the bulk zone was greater than that of fertilizer (Figure 7.5), confirming the dissimilarity of the relative abundances between growing medium harboring different plants and the no plant treatment. These results are unexpected, as the bulk zone is defined as the zone of growing medium that is not influenced by root secretions or in other words is not influenced by the plant. We expected to see a fertilizer effect, but plant effect was stronger than fertilizer effect. The organic fertilizer, however, was distinctly different from the no fertilizer treatment and struvite. The organic nitrogen mineralization relies directly on the microbial nitrogen conversion and results in a release of ammonia, while the use of struvite depends mainly on physico-chemical environment. In our study, samples were taken at a distance of more than

10 mm from the root zone. Researchers have shown that the influence can be up to 10 mm (Hartmann et al. 2008; Jones et al. 2009; Niu et al. 2012), however, our results indicate the plants can influence the microbial community composition at a distance of more than 10 mm in the organic growing medium.

MFA showed a clear separation of the bacterial abundance between samples of the growing medium with lupine, tomato and no plant and this was the case for the bulk zone. When plants start rooting in the growing medium, they immediately encounter the microbial community associated with the growing medium, resulting in the establishment of a microbial rhizosphere community closely interacting with the plants and a microbial community distinct from the rhizosphere (Gschwendtner et al. 2016). These results are in agreement with Berg and Smalla (2009) who indicate that plant species and soil or growing medium type cooperatively shape the structure of the microbial community in the rhizosphere.

As suggested by Baudoin et al. (2002) time is also an important factor. Mentioned study indicates that the differences between bulk and rhizosphere soil responses are more pronounced after 4 weeks (Day 30) compared to plants that are 2 weeks old. Moreover, microbial community at the seedling stage of *Arabidopsis* was distinct from other developmental time points (Chaparro et al. (2014). In this way, our analysis confirmed the dissimilarity of the bacterial community associated with the growing medium harboring different plants (Figures 7.5, Figure 7.6 and Figure 7.7). These results are in agreement and complementary to a study of Girvan et al. (2003), however this study did not include chemical factors that were shown in the present study to correlate with the microbial community structure in soilless culture systems. In addition, as root exudation changes during plant growth development, the sampling time might be another critical factor in the experimental design.

Most investigations of the rhizosphere have relied on collecting soil which remains adhered to plant roots after the plant has been removed from soil ('pull and shake systems', (Duineveld et al. 2001; Katznelson et al. 1948)). Using this sampling strategy, it has been relatively easy to collect sufficient soil for comparative chemical and microbiological analyses but it is likely that rhizosphere and bulk soil samples have been mixed thus integrating across several niches. This indicates that there are major problems in sampling and the identification of true rhizosphere effects remains difficult. This limits our ability to assess the extent to which the plant selects for and regulates its own rhizosphere community (O'donnell et al. 2001).

In this study, the community composition in the bulk zone of the growing medium in combination with a plant and no plant was distinctly different indicating that plants can influence the microbial community composition at a distance of more than 10 mm in organic growing media.

4.2 Plants drive microbial community composition in organic growing media blended with recovered nutrients

MFA revealed that the impact of plant, time and sterilization on the microbial community in the bulk zone was greater than that of fertilizer (Figure 7.5). In addition, Figure 7.6 and Figure 7.7 showed that the plant effect is determinant on the differences in the relative abundances of the communities in the rhizosphere, confirming the dissimilarity of the relative abundances between growing medium harboring different plants.

A total of 11 chemical variables were monitored over time for the different soilless culture systems (Supplementary table 7.3). Throughout the study, no major anomalies concerning plant growth occurred and plant performance was affected in similar way with respect to the fertilizer used. The use of struvite resulted in a decrease of the leaf area (25-26%), fresh weight (25-28%) and dry weight (22-28%) independent of the plant used compared to the organic fertilizer. When no fertilizer was applied, plant response was different. The no fertilizer treatment resulted in a decrease of the leaf area by 90% for tomato and 40% for lupine. In addition the fresh weight was decreased by 93% for tomato and 43% for lupine and a decrease of the dry weight by 92% for tomato and 33% for lupine compared to the organic fertilizer, indicating that lupine behaves differently with respect to nutrient acquisition compared to tomato under low nutrient conditions. This might be explained by the fact that the highest nodulation and nodule activity at zero or a low growing medium nitrogen content is obtained when the seed reserves are available in amounts that are sufficient for vigorous plant growth during the first weeks of plant growth (Marschner 2011). The cotyledons of leguminous seeds, such as lupine, contain large amounts of reserve proteins (Garnczarska et al. 2007).

As indicated by Pieterse et al. (2016) root interior, root surface, soil or growing medium close to the root surface, and unplanted bulk soil or growing medium have distinct microbiomes (Edwards et al. 2015) and that soil and growing medium serves as an important microbial reservoir for microbial community assembly in the phyllosphere (Bai et al. 2015). Underlying principles of plant-microbiome interactions have been nicely described in the microbial market theory (Werner et al. 2014), in which economic market characteristics, such as exchange of commodities between trading partners (plant versus microbe and microbe versus microbe), 'price wars' (best return of investment), supply and demand dynamics, and elimination of the competition, drive community assembly of microbiota at the root-growing medium interface.

Plant growth is affected by fertilizer used and it is acknowledged that additional N supply affects soil microbial community structure (Ai et al. 2012; Gschwendtner et al. 2016). During the vegetation period, photosynthetically assimilated CO_2 is released by the plants via rhizodeposition (root exudates, mucilage, enzymes and sloughed root cells) into the growing medium. The microbial community in the rhizosphere benefits from the surplus of easily degradable carbon sources provided by plants and in turn enhance plant growth and health via nutrient mobilization, production of plant growth hormones, induction of systemic resistance and controlling pathogens (Raaijmakers et al. 2009). Due to this close plant-microbe interaction, plants are able to select for a certain rhizosphere microbial community, potentially through its root exudates (Gschwendtner et al. 2016). Plants are known to distribute assimilates from photosynthesis to actively growing parts, therefore the roots have a large amount of carbon to exudate during the vegetative phase.

In many cases, changes in evenness occur with little or no changes in species richness (Wilsey and Potvin 2000). Research that investigates species evenness in relation to productivity is scarce. Decreases in plant species evenness may have an indirect lowering effect on plant productivity (Wilsey and Potvin 2000). Naeem and Li (1997) hypothesized that diverse communities are more productive because a greater proportion of light is captured by the plant community as a whole. Hooper (1998) indicated that a higher productivity can be explained by a greater complementary use of resources in space and time and thus a shift in community interactions from high competition to weak competition. Wilsey and Potvin (2000) showed that differences in the interception of light were probably more important than nutrient uptake in explaining the plant community responses to evenness. It was

indicated that nutrient storage by plants increase when biodiversity increased (Balvanera et al. 2006). We observed in agreement with Balvanera et al. (2006) an increase in leaf area, fresh weight and dry weight in combination the organic fertilizer compared to the struvite. On the contrary, the lowest leaf area, fresh weight and dry weight were found in combination with the no fertilizer treatment although the highest values for biodiversity and evenness were detected in the no fertilizer treatment. In addition, research indicates that higher species richness generally leads to higher community functionality (Wittebolle 2009), such as mineralization and nitrification, however mentioned functionality depends largely on external factors, such as the addition and the type of fertilizers used.

Indeed, fertilizers restore and optimize the physico-chemical condition of the growing medium, improving the availability of nutrients, controlling pH and osmotic potential in the rhizosphere (Sonneveld and Voogt 2009a). Fertilizers are also of utmost importance for optimal plant growth. Our results suggest that ammonium struvite and organic fertilizer can deliver plant available nutrients. However, the use of struvite as a nitrogen source, resulted in a significant decrease of the leaf area, fresh and dry weight in both tomato and lupine compared to an organic fertilizer. Our results are in agreement with Li and Zhao (2003), who found that the germination and growth of the selected vegetables in the pots with struvite showed significantly better plant performance than those in the pots without struvite as a control. The effectiveness of struvite (insoluble phosphate precipitated from wastewater) as a P source was also tested by Gonzalez Ponce and De Sa (2007). They compared struvite to that of phosphate rock (insoluble), monoammonium phosphate (soluble) and calcium superphosphate (soluble) by providing each to perennial ryegrass (*Lolium perenne)* growing in pots. They concluded that P nutrition with struvite was as effective as with any other source (Cabeza et al. 2011; Plaza et al. 2007). We, however, used struvite as a nitrogen source. The better performance of the struvite in mentioned study of Li and Zhao (2003) can be explained by the fact that the solubilisation rate of the struvite based fertilizer was lower compared to our results. Even after 105 days, the total N released was in the range of 9.6–23.2% depending on the formulation. The slow-releasing properties of struvite confirm the earlier findings of Rahman et al. (2011). We, however, found higher releasing rates of about 31% after 30 days. These increased solubilisation rates can be explained by the fact that we used a more acidic growing medium and lower temperatures (18°C night and 24°C during day). As shown by Latifian et al. (2012), the percentages of nutrients released from struvite were slightly higher in formulations with starch addition, suggesting that microorganism may alter the solubility of struvite, under sterile conditions this effect was not found. These finding, indicate that the release of ammonium from struvite is also a combination of a biological and chemical driven process.

The availability of ammonium from organic fertilizers relies on microbial activity, while release of ammonium from struvite is a combination of a chemically and biologically driven process (Achat et al. 2014). Indeed struvite resulted in a steadily increase of the ammonium concentration over time (Britto and Kronzucker 2002; Qin et al. (2011)). The positive correlation (Supplementary table 7.9) between ammonium and phosphorus concentrations may indicate that decreased plant performance in our trial was caused due to ammonium toxicity coupled with low potassium concentrations (Britto and Kronzucker 2002). Ammonium concentrations were two (time point 1) to three times (time point 2) higher in combination with struvite in comparison with the organic fertilizer treatment. In addition, the nutrient dynamics in the growing medium showed increased nitrate concentrations in combination with the organic fertilizer in comparison to struvite. These results indicate higher nitrification activity, in combination with the organic fertilizer compared to struvite. Combination of both can results in high

or even toxic ammonium concentrations explaining decreased plant performance. These results indicate that plants rather than fertilizers drive rhizosphere microbial community composition in organic growing media blended with recovered nutrients.

4.3 The rhizospheric microbial community is more even, diverse and distinctly different from the bulk zone microbial community

As shown in table 7.5 location, i.e. rhizosphere or bulk zone had no effect on total species, evenness and diversity. MFA was also used to indicate significant differences in relative bacterial abundance as a result of plant, location (bulk or rhizosphere), time and fertilizer. The confidence ellipse of the rhizosphere and the bulk zone shows significant separation of the microbial community by their location (Figure 7.6), which might be contradictory to the first finding. Indeed, the number of species (richness), the relative abundances of each of these species (evenness) and the pool of species (diversity) is the same in bulk and roots. However, the beta diversity measures were significantly different. This means that there are differences in species composition between the two locations; this is to say that not all the same species are present in both environments. This might indicate that the number of species is the same, but not the way they are distributed within time points, within fertilizers and within plants. Indeed, we have indications that the species composition between bulk zone and rhizosphere is different (Supplementary table 7.7 and 7.8).

Smalla et al. (2001) analyzed the bacterial rhizosphere communities of three host plants of the pathogenic fungus *Verticillium dahliae*, field-grown strawberry (*Fragaria ananassa* Duch.), oilseed rape (*Brassica napus* L.), and potato (*Solanum tuberosum* L.). Mentioned study, aimed to determine the degree to which the rhizosphere effect is plant dependent and whether this effect would be increased by growing the same crops in two consecutive years. Based on DGGE profiles that mainly reflect the evenness of populations in an environmental sample, Smalla et al. (2001) found an increased relative abundance of some populations in the vicinity of the roots for all three plants compared to the bulk zone. Samples were taken during 5 months after sowing or planting. In contrast, Duineveld et al. (2001) observed no differences or only minor differences between bulk zone and the rhizosphere of chrysanthemum plants grown in pots in a growth chamber. Samples were taken 2, 4, 6, and 10 weeks after planting. No differences between bulk and rhizosphere patterns were found in a study by Normander and Prosser (2000) for barley grown in pots in a growth chamber during the first 36 days after sowing. Plant root exudates are differentially produced at distinct development stages to orchestrate rhizosphere microbiome assemblage (Chaparro et al. 2014). As shown by Aulakh et al. (2001), shoot and root biomass were positively correlated to carbon exudation suggesting that exudation is driven by plant biomass. Consequently, seedlings exude the lowest amount of exudates, which might explain the fact that no differences were found in microbial community composition based on species richness, evenness and diversity. As the plant grows, the exudation rate increases until it sets flowers, and as it matures the rate decreases again. Indeed, rhizospheric bacterial communities of a wide range of plants (i.e., *Arabidopsis*, *Medicago*, maize, pea, wheat and sugar beet) change according to plant developmental gradient (Baudoin et al. 2002; Houlden et al. 2008; Micallef et al. 2009; Mougel et al. 2006). Consequently, plant development stage is an important factor influencing rhizospheric microbial community composition.

Our results show, that the no fertilizer treatment has a higher species richness (total species), diversity (Shannon, Fisher's alpha, Simpson and Inverse Simpson indices), and evenness followed by the organic

fertilizer and finally the struvite treatment in the rhizosphere. As shown by Marschner et al. (2001) in a short-term experiment (15–22 days) with cucumber and barley growing in a N deficient or a P deficient soil, the bacterial community structure in the rhizosphere was affected by soil type and fertilization but not by plant species. Indeed, under these conditions, the rhizosphere effect might be masked by the continued presence of dominant groups already present in the growing medium. Many dominant bacterial groups might be dormant under particular rhizosphere conditions, but their presence would still be detected by DNA-based analyses. Under P and N deficient conditions plant shoot and root biomass is decreased compared with optimal conditions and consequently, root exudation and influence on rhizospheric microbial community composition is decreased.

Based on the MFA results, and taking all the effects into account, we observed that bacterial abundance in the rhizosphere were significantly different regardless of fertilizer supplementation. In the bulk zone, on the contrary, this effect was not observed. In addition, MFA showed, that the microbial community in the rhizosphere is getting more specific over time, indicated by the decreased variations in the bacterial relative abundances in the rhizosphere over time. As a consequence, differences in rhizosphere microbial community composition compared to the bulk zone are mainly influenced by plant and time point, i.e. plant age.

4.4 Plants affect abundance and activity of rhizospheric microbial communities in combination with tomato and fertilizer used?

The relative AOB abundance (copy number ratio of bacterial *amoA*: total bacteria) was significantly impacted by fertilizer type ($P < 0.05$), but not by location, i.e. rhizosphere or bulk zone or interaction of the factors. The highest relative AOB abundance was found in combination with the organic fertilizer compared to struvite. The relative NOB abundance (ratio of the *Nitrobacter+Nitrospira*:total bacteria) was not significantly influenced by fertilizer used and location (Table 7.4). *Nitrobacter* are higher in absolute and relative numbers in comparison to *Nitrospira* within the NOB community in the organic growing medium. No significant shifts in the relative *Nitrobacter/Nitrospira* ratio associated with location, fertilizer treatment or the interaction of both were detected (Table 7.4).

Functionality tests revealed that ammonia oxidation activity was significantly higher ($P < 0.05$) in the bulk zone (60 ± 4 µg N g^{-1} growing medium d^{-1}) in comparison with the rhizosphere (37.0 ± 0.4 µg N g^{-1} growing medium d^{-1}). Hence, the potential activity of the ammonia oxidizing organisms in the rhizosphere was 38% lower in comparison to the bulk zone. Ammonia oxidation was significantly lower ($P < 0.05$) when no fertilizer was supplied (32 ± 3 µg N g^{-1} growing medium d^{-1}), but no differences between the organic fertilizer (37 ± 3 µg N g^{-1} growing medium d^{-1}) and struvite (37 ± 2 µg N g^{-1} growing medium d^{-1}) were found. Plant roots stimulate rhizosphere heterotrophs, activate mineralization of organic nitrogen and exudated nitrogen sources (el Zahar Haichar et al. 2014) leading to increased ammonium fluxes in the rhizosphere (Thion et al. 2016). Root exudates are part of the rhizodeposition process, which is the major source of soil organic carbon released by plant roots. These exudates include amino acids, organic acids, sugars, phenolic substances and other secondary metabolites (el Zahar Haichar et al. 2014).

On the other hand uptake of ammonium (Britto and Kronzucker 2002; Marschner 2011) and organic-derived N (Näsholm et al. 2000; Näsholm et al. 2009) by the roots suggests a direct competition for ammonium with the AOB in the rhizosphere or indirectly due to a reduction of the nitrogen mineralization resulting in decreased ammonium concentrations (Thion et al. 2016). In addition, the

cation exchange capacity of the growing medium (GB) may also influence the ammonium availability due to abiotic immobilization. Abiotic immobilization capacity is assumed to be equal throughout the whole growing medium (GB). In general microorganisms are superior to plants with respect to the competition for nitrogen (Näsholm et al. 2009). Therefore, one would expect a higher AOB abundance in the rhizosphere compared to the bulk zone. This was not confirmed in our study. On the contrary, we found that AOB abundance was not affected by location, which is in agreement with Thion et al. (2016), suggesting that AOB did not benefit from increased N supply from roots. Competition relies on the ammonium availability and diffusion of the different N forms in the growing medium and may be significant in low N systems (Schimel and Bennett 2004). In addition, low availability of ammonium limits both the nitrification rates and the nitrifier population size (Shi and Norton (2000). The AOB abundance was not influenced by location (rhizosphere and bulk zone), but by fertilizer used. Lower AOB numbers were observed when no fertilizer was applied, suggesting that AOB benefit from the increased N supply through exudation from fertilizers, efficiently competing for the ammonia with other microorganisms and/or with the tomato plant (Thion et al. 2016). Affinity for ammonium might be a key characteristic in the rhizosphere. Microorganisms with a high affinity for nutrients, such as ammonium, in general have low growth rates and are classified as K-strategists. Microorganisms, on the contrary, with a low affinity have in general a high growth rate and are classified as r-strategist (Fontaine et al. 2003). These results might indicate, that the rhizosphere is colonized by mainly K strategists being able to compete with the plants. The bulk zone, on the contrary, is mainly colonized by r-strategists and are present due to the decreased competition with the plant and showing a higher growth rate and maximum ammonia oxidation activity.

Non-invasive pH measurements with the planar optodes showed an increase of the rhizosphere pH and the presence of nitrate at the third time point with the organic fertilizer. Indeed, uptake of nitrate results in excess uptake of anions over cations, net uptake of protons and thus an increase in the rhizosphere pH. Furthermore the assimilation of nitrate in the root tissue is accompanied by the production of OH- ions and may therefor contribute to some extent to rhizosphere alkalization by the release of OH- ions ion the rhizosphere for intracellular pH stabilization (Marschner 2011). These results might indicate that the ammonia oxidizing bacteria are equal or better competitors for ammonia than the tomato plants. Higher microbial activity in the rhizosphere, including organic nitrogen (N) mineralization, may stimulate ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA), while ammonia uptake by plants may favor AOA, considered to prefer lower ammonia concentration and while high ammonia concentrations may favor AOB. Ammonium is a weak acid with a pKa 9.25. This means that at pH(H_2O) 9.25 the ratio between ammonium and ammonia in a solution equal is to 1:1. Significant dissociation takes place at pH(H_2O) values higher than 6.25. At this value approximately 1‰ of the ammonium is dissociated to ammonia. At pH(H_2O) 7,25 this is 1% and at pH(H_2O) 8,25 this is 10%. This suggests that AOB are better competitors for ammonia/ammonium than plants under fertilized conditions (100 mg N L^{-1} growing medium), under N deficient conditions, on the contrary, plants are the better competitors.

Baudoin et al. (2003) demonstrated that the bacterial densities are significantly increased in the rhizosphere in comparison to the bulk zone, which is also confirmed by our results (Table 7.4). *Nitrobacter* seem to be the key players in absolute and relative numbers in comparison to *Nitrospira* within the NOB community in the organic growing medium. Nogueira and Melo (2006) suggested that *Nitrobacter* is a superior competitor when resources are abundant, while *Nitrospira* thrive under conditions of resource scarcity. We found no shifts in the relative Nitrobacter/Nitrospira ratio

associated with location and fertilizer treatment, indicating interactions between ammonia oxidizers and nitrite oxidizers (Wang et al. 2015).

Ammonia oxidation rate, however, was lower in the rhizosphere in comparison to the bulk zone, indicating inhibition of the ammonia oxidation in the rhizosphere or even stimulation of ammonia oxidation in the bulk zone. Acidification of the rhizosphere might inhibit nitrification rates (Falkengren-Grerup 1995; Haynes and Goh 1978). We on the contrary found increased pH values in the rhizosphere. Plants roots can release compounds to suppress nitrification (biological nitrification inhibition) (Subbarao et al. 2007b). Inhibition of nitrification is likely to be part of an adaptation mechanism to conserve and use N efficiently in natural systems that are N limiting (Lata et al. 1999; Subbarao et al. 2007a), however this was not shown in soilless culture systems in combination with organic growing media and tomato plants. Crops including rice (*O. sativa*), maize (*Z. mays*), wheat and barley (*H. vulgare*) were found to lack nitrification inhibition capacity in their root systems during initial screening studies (Subbarao et al. 2007a). In addition, nitrification inhibition is stimulated in the presence of ammonium (Subbarao et al. 2007b).

Our results challenge the theory that microorganisms are superior to plants with respect to competition for nitrogen in soilless culture systems in combination with organic growing media. Tomato plants seems to be able to influence or even modulate the nitrification activity in the rhizosphere and the highest relative AOB abundance was found in combination with the organic fertilizer compared to struvite.

5. Conclusion

We investigated the microbial community in soilless culture systems in combination with two plant, two different fertilizer over time, in terms of microbial community structure and functionality, i.e. ammonia oxidation rate. We showed that:

- The community composition of the growing medium in combination with a plant and no plant was distinctly different and this could be attributed to differences in chemical factors in soilless culture systems.
- The use of organic fertilizer in combination with organic growing media result in a even and diverse microbial community, however species richness was not affected. Inorganic fertilizer, such as struvite results in microbial communities with a lower evenness and diversity.
- Rhizosphere microbial community composition was influenced by plant development stage and by the amount and type of fertilizer used in soilless culture systems.
- No differences in AOB abundance between the rhizosphere compared to the bulk zone were found challenging the theory that microorganisms are better competitors for nitrogen than plants.

6. Acknowledgements

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Supplementary information

Supplementary table 7.1: Overview of the primer concentration, volume and annealing temperature used for the specific bacterial groups

	Primer concentration	*Volume*	*Annealing temperature*
16S Bacteria	*500 nM*	*0.5µL*	*57°C*
16S Nitrobacter	*300 nM*	*0.3µL*	*64°C*
16S Nitrospira	*600 nM*	*0.6 µL*	*54°C*
amoA Bacteria	*500 nM*	*0.5 µL*	*59°C*
16S Archaea	*800 nM*	*0.8 µL*	*60°C*
AmoA archaea	*500 nM*	*0.5 µL*	*59°C*

Supplementary table 7.2: Overview of the target bacteria and the template used

Target	Template (stD curve)		reference
16S bacteria	PCR product *E. coli* ATCC 10536	519F	
		907R	
16S Archaea	PCR product *Archaeoglobus fulgidus* DSM4304	Arch349F	
		Arch806R	
16S Nitrospira	PCR product from 16S Nitrospira (environmental sludge) clone	NSR 1113F	(Dionisi et al. 2002)
	Company DANIS (manure treatment)	NSR 1264R	(Graham et al. 2007)
16S Nitrobacter	PCR product from 16S Nitrobacter (nitrifying bacterial consortium) clone	Nitro-1198F	
	ABIL (AVECOM)	Nitro-1423R	
amoA Bacteria	PCR product from amoA (*N. europaea*) clone	amoA 1F	(Rotthauwe et al. 1997)
		amoA 2R	
amoA Archaea	PCR product from fosmid 54D9	crenamo23f	
		crenamo616r	(Treusch et al. 2005)

Supplementary table 7.3: Influence of fertilizer type (no fertilizer -NOF, organic fertilizer – ORG and ammonium struvite-STR) on the nutrient dynamics in non-sterile organic growing media with plants (LUPINE and TOMATO) and without plants (NOPLANT) a plant in function of time. n= 5. NS = not significant effect SEM= standard error of the mean, $P<0.05$.

Variable	Tpt	Plant	Fertilizer			SEM	Effect and present P value						
			NOF	ORG	STR		Plant	Fertilizer	Time point	Plant*Fertilizer	Plant*Time point	Fertilizer*Time point	Plant*Fertilizer*Time point
pH(H_2O)	1	NOPLANT	5.5	5.7	5.6	0.03	0.0001	0.0002	<0.0001	0.0009	<0.0001	<0.0001	NS
		LUPINE											
		TOMATO											
	2	NOPLANT	6.2	6.2	6.1								
		LUPINE	6.7	6.3	6.1								
		TOMATO	5.9	5.7	6.0								
	3	NOPLANT	6.0	5.6	5.6								
		LUPINE	6.1	5.3	5.4								
		TOMATO	5.7	5.5	5.6								
Conductivity (µS/cm)	1	NOPLANT	93.3	127.3	83.0	6.7	0.01	<0.001	<0.001	NS	0.02	0.001	NS
		LUPINE											
		TOMATO											
	2	NOPLANT	98.7	130.9	181.5								
		LUPINE	99.2	158.0	231.2								
		TOMATO	123.5	199.0	194.4								
	3	NOPLANT	141.3	251.2	284.4	6.8							
		LUPINE	119.2	259.0	317.6								
		TOMATO	189.4	327.0	332.4								
NO_3-N (mg.L^{-1})	1	NOPLANT	0.0	0.0	0.0	1.03	0.0076	<0.001	<0.001	NS	<0.001	<0.001	<0.001
		LUPINE											
		TOMATO											
	2	NOPLANT	0.0	2.5	0.0	1.03							
		LUPINE	0.0	0.0	0.0								
		TOMATO	0.0	23.3	0.0								
	3	NOPLANT	0.0	13.8	13.1	1.05							
		LUPINE	0.0	36.4	26.9								
		TOMATO	0.0	3.1	0.0								
NH_4^+-N (mg.L^{-1})	1	NOPLANT	1.7	18.4	42.1	1.4	NS	0.001	NS	0.03	<0.001	<0.001	0.003
		LUPINE											
		TOMATO											
	2	NOPLANT	3.6	20.1	49.4	1.4							

		LUPINE	5.8	22.8	70.1								
		TOMATO	5.0	3.6	42.1								
		NOPLANT	0.4	16.3	54.7								
	3	LUPINE	1.6	3.0	50.7	1.4							
		TOMATO	1.9	0.5	74.3								
		NOPLANT											
	1	LUPINE	18.7	27.3	203.1	4.9							
		TOMATO											
		NOPLANT	12.9	21.6	150.1								
P ($mg.L^{-1}$)	2	LUPINE	12.4	25.3	224.7	4.9	0.0008	<0.001	<0.0001	0.003	0.006	<0.001	0.008
		TOMATO	10.3	18.2	126.8								
		NOPLANT	11.5	19.7	206.1								
	3	LUPINE	29.5	23.3	331.0	5.0							
		TOMATO	9.5	18.3	290.7								
		NOPLANT											
	1	LUPINE	111.5	160.0	110.0	4.2							
		TOMATO											
		NOPLANT	100.8	156.8	109.8								
K ($mg.L^{-1}$)	2	LUPINE	105.5	178.1	122.5	4.2	NS	<0.0001	0.03	NS	0.01	NS	NS
		TOMATO	103.5	196.5	128.5								
		NOPLANT	117.5	218.6	139.4								
	3	LUPINE	100.3	165.5	113.0	4.3							
		TOMATO	116.0	187.5	137.5								
		NOPLANT											
	1	LUPINE	812.0	807.5	782.0	10.9							
		TOMATO											
		NOPLANT	855.3	758.0	759.8								
Ca ($mg.L^{-1}$)	2	LUPINE	976.5	840.6	726.5	10.9	0.03	<0.0001	NS	<0.0001	NS	NS	0.0007
		TOMATO	743.5	970.0	842.5								
		NOPLANT	830.5	813.1	735.6								
	3	LUPINE	933.1	803.5	734.0	11.2							
		TOMATO	714.5	1007.8	841.5								
		NOPLANT											
	1	LUPINE	270.5	275.5	386.0	4.2							
		TOMATO											
		NOPLANT	297.3	269.3	397.0								
Mg ($mg.L^{-1}$)	2	LUPINE	327.0	297.7	430.0	4.2	0.0002	<0.0001	0.002	<0.0001	0.0003	NS	NS
		TOMATO	265.0.0	323.5	411.0								
		NOPLANT	286.0	254.4	378.0								
	3	LUPINE	336.2	295.5	442.5	4.3							
		TOMATO	259.0	326.8	415.0								
		NOPLANT	102.8	194.4	98.5								
	1	LUPINE				4.1							
SO_4 ($mg.L^{-1}$)		TOMATO					<0.0001	<0.0001	<0.0001	NS	<0.0001	0.02	0.03
		NOPLANT	87.9	143.7	83.9								
	2	LUPINE	59.0	125.9	47.2	4.1							

		TOMATO	103.8	136.4	112.3							
		NOPLANT	106.9	166.2	101.2							
	3	LUPINE	53.0	86.9	67.3	4.2						
		TOMATO	112.2	229.9	109.8							
		NOPLANT	54.0	56.5	56.5							
	1	LUPINE				1.0						
		TOMATO										
		NOPLANT	46.0	46.3	45.3							
Na (mg.L^{-1})	2	LUPINE	47.5	55.1	52.5	1.0	<0.0001	0.001	<0.0001	NS	<0.0001	NS	NS
		TOMATO	40.0	43.0	45.0							
		NOPLANT	45.0	43.9	51.4							
	3	LUPINE	2.6	4.5	3.5	1.0						
		TOMATO	48.5	68.1	68.0							
		NOPLANT	99.5	99.2	100.8							
	1	LUPINE				6.5						
		TOMATO										
		NOPLANT	95.9	83.9	88.3							
Cl (mg.L^{-1})	2	LUPINE	84.8	95.9	83.5	6.5	<0.0001	NS	NS	NS	<0.0001	NS	NS
		TOMATO	150.4	162.9	140.7							
		NOPLANT	130.3	201.1	145.2							
	3	LUPINE	0.4	0.6	0.5	6.9						
		TOMATO	180.9	259.7	186.7							

Supplementary table 7.4: Influence of fertilizer type (no fertilizer -NOF, organic fertilizer – ORG and ammonium struvite-STR) and the location (rhizosphere versus bulk) on the different ratio's in non-sterile organic growing media with tomato. n= 8. NS = not significant effect. Numbers are μ±σ; LOD= limit of detection was 10^3 for amoA bacteria, 16S Bacteria, 16S Archaea, 16S Nitrobacter and 16S Nitrospira and 10^2 for the archaeal amoA gene copy number.

	NOF		ORG		STR		Effect		
	bulk	rhizosphere	bulk	rhizosphere	bulk	rhizosphere	Fertilizer	Location	Fertilizer*location
16S Nitrobacter/ total 16S bacteria	2.20±1.80%	2.44±1.80%	1.15±1.37%	2.36±0.99%	1.68±1.31%	1.81±1.36%	NS	NS	NS
16S Nitrospira/ total 16S bacteria	0.03±0.03%	0.02±0.02	0.03±0,03%	0.05±0.03%	0.06±0.04%	0.03±0.03%	NS	NS	NS
amoA Bacteria /16S bacteria	<LOD[a]	<LOD[a]	2.59±1.19%[b]	2.7±1.15%[b]	0.01±0.02%[a]	0.34±0.89%[a]	<0.001	NS	NS
16S Nitrobacter/16S Nitrospira	29.2±7.8	32.3±7.0	24.5±7.1	25.5±5.6	31.8±6.3	NS	NS	NS	NS

Supplementary table 7.5: Correlations between relative bacterial abundances and chemical characteristics in the bulk growing medium and plant, fertilizer, condition (sterile versus non-sterile) across time points, indicated by the Multiple Factor Analysis. Dimensions of the MFA can be described by the categorical variables included in the analysis. For each categorical variable (fertilizer, condition, plant, and time point), a one-way analysis of variance was performed with the coordinates of the samples on the axis, explained by the time point or growing medium type. Then, for each level of the category (i.e. time point 1, time point 2 or time point 3 or fertilizer, condition and plant), a Hotelling T^2-test was used to compare the average of the category with the general average (using the constraint P i αi = 0, αi = 0). For instance, the coordinates of the relative abundance of genera "x" at time point 2 were compared with the average coordinates of the relative abundance of genera "x" a. The P value associated to this test is transformed to a normal quantile to assess whether the mean of the category is significantly less or greater than 0. Negative values indicate negative correlations.

Dimension	Variance	Descriptor	Estimate (R^2)	P value	Taxon	Correlation	*P* value
					Acidobacteria_Gp1	0.683	0
					Rhodoferax	0.635	0
					unclassified.Gammaproteobacteria	0.634	0
					Bdellovibrio	0.624	0
		Non sterile	0.54	0.0000021	*Granulibacter*	0.623	0
		Lupine	0.71	0.0000288	*Actinobacterium*	0.619	0
		Tpt2	0.49	0.0000392	*Methylovirgula*	0.606	0
		struvite	0.39	0.0114	*Roseiarcus*	0.584	0
					unclassified.Rhodospirillaceae	0.575	0
					Sinobacteraceae	0.573	0
1	13.51%				*Acidocella*	0.558	0
					Unclassified.Solirubrobacter	0.549	0
					Actinomadura	0.535	0
					Frankineae	0.525	0
					Cryptosporangium	0.507	1.00E-07
					Actinospica	0.492	3.00E-07
					X3_genus_incertae_sedis	0.488	4.00E-07
					unclassified.Acidimicrobiaceae	0.483	6.00E-07
					Conexibacter	0.481	7.00E-07
					Spirochaeta	0.472	1.20E-06

Acidothermus	0.450	4.10E-06
Thermomonosporaceae	0.446	5.10E-06
Acidobacteria_Gp14	0.439	7.50E-06
Acidisphaera	0.427	1.43E-05
Thermoanaerobacteraceae	0.420	2.03E-05
Rhodanobacter	0.418	2.17E-05
Wandonia	0.411	3.05E-05
unclassified.Verrucomicrobia	0.408	3.54E-05
unclassified.Rubrobacteria	0.398	5.70E-05
Anaeromyxobacter	0.378	0.000144
Aquicella	0.370	0.0002
Microbacterium	0.350	0.000461
unclassified.Actinomycetales	0.341	0.000671
Frankiaceae	0.323	0.001327
stella	0.314	0.001774
Alkanibacter	0.313	0.001858
Magnesium	0.312	0.001906
Rhodobacter	0.310	0.002089
Verrucomicrobium	0.308	0.00222
uncultured.Thermomicrobia	0.301	0.002876
Phosphorus	0.297	0.003234
Rhizomicrobium	0.294	0.003641
Nocardia	0.291	0.003988
stigmatella	0.290	0.00411
Armatimonas_Armatimonadetes_gp1	0.288	0.004351
Sorangiineae	0.283	0.005116
Aciditerrimonas	0.273	0.006935
Prosthecobacter	0.271	0.007502
pH(H2O)	0.264	0.009342
Porphyrobacter	0.261	0.009935

Gemmatimonas	0.260	0.010359
Acidobacteria_Gp3	0.254	0.01228
unclassified.Bacteroidetes	0.253	0.012764
unclassified.Roseateles	0.251	0.013633
Mucilaginibacter	0.249	0.014204
Nitrosospira	0.245	0.016063
unclassified.Myxococcales	0.231	0.023252
Mycobacterium	0.226	0.026278
Methylocystis	0.213	0.036359
Paenibacillus	-0.285	0.00477
Bradyrhizobium	-0.291	0.004022
Singulisphaera	-0.292	0.003868
Chitinophaga	-0.293	0.003711
Rhodococcus	-0.306	0.002363
Uncultured.Carnobacterium	-0.309	0.002181
Methylophilus	-0.318	0.00156
Cupriavidus	-0.322	0.001342
Methylobacterium	-0.326	0.001176
Rummeliibacillus	-0.327	0.001105
Pedobacter	-0.328	0.001102
Escherichia_Shigella	-0.328	0.001073
Spirosoma	-0.346	0.000544
Hyphomicrobium	-0.353	0.000406
Pullulanibacillus	-0.370	0.000201
Ca	-0.388	9.04E-05
Burkholderia	-0.394	7.01E-05
Bacillus	-0.400	5.32E-05
Achromobacter	-0.403	4.55E-05
Massilia	-0.424	1.64E-05
Leifsonia	-0.426	1.49E-05

					Labrys	-0.436	8.80E-06
					TM7_genus_incertae_sedis	-0.440	7.00E-06
					Tardiphaga	-0.453	3.50E-06
					EC	-0.466	1.70E-06
					Niastella	-0.471	1.30E-06
					Pseudomonas	-0.481	7.00E-07
					Afipia	-0.486	5.00E-07
					Beijerinckia	-0.500	2.00E-07
					Kaistia	-0.507	1.00E-07
					Rhizobium	-0.538	0
					Sphingomonas	-0.545	0
					Bosea	-0.559	0
					Nocardioides	-0.625	0
					Cl	-0.707	0
					SO4	-0.708	0
					K	-0.738	0
					NH4	0.8305	0
					P	0.8280	0
		struvite	1.138182	0	*EC*	0.8012	0
		tpt3	0.273812	0.015029	*Mg*	0.7558	0
					Frankiaceae	0.3498	0.000475
					unclassified.Myxococcales	0.3374	0.000772
					Na	0.3343	0.000872
2	10.96%				*unclassified.Rubrobacteria*	0.3120	0.001964
					Roseiarcus	0.2859	0.004736
					Acidobacteria_Gp3	0.2820	0.005366
					Verrucomicrobium	0.2755	0.006583
					Cl	0.2578	0.011211
					Conexibacter	0.2568	0.011526
					unclassified.Firmicutes	0.2555	0.011964

		Actinobacterium	0.2484	0.014659
		Frankineae	0.2476	0.01497
		Unclassified.Solirubrobacter	0.2324	0.022662
		unclassified.Opitutae	0.2264	0.026485
		Microbacterium	0.2152	0.035194
		Acidobacteria_Gp1	0.2094	0.040581
		X3_genus_incertae_sedis	0.2071	0.042844
		Rhizomicrobium	0.2017	0.04873
		Pasteuria	-0.202	0.048416
		Polyangium	-0.203	0.046488
		Uncultured.Fibrobacter	-0.205	0.045009
		Demequina	-0.208	0.04141
		Archangium	-0.212	0.037355
		Dyadobacter	-0.215	0.034771
		Spirosoma	-0.224	0.028012
		Uncultured.Rhizobium	-0.224	0.027931
		Caulobacter	-0.226	0.026435
		Bordetella	-0.229	0.024609
		Dongia	-0.243	0.01684
		Mesorhizobium	-0.245	0.016088
		unclassified.Deltaproteobacteria	-0.247	0.015213
		Opitutus	-0.252	0.01289
		Hirschia	-0.273	0.006978
		Iamiaceae	-0.283	0.005103
		Flavobacterium	-0.287	0.004511
		Ohtaekwangia	-0.298	0.003173
		Verrucomicrobiaceae	-0.302	0.002747
		Peredibacter	-0.308	0.002219
		pH(H2O)	-0.415	2.52E-05
3	8.0%	*X3_genus_incertae_sedis*	0.5660	0

tomato	0.584367	4.30E-06
organic	0.456697	0.000167
tpt2	0.270512	0.004737

SO4	0.5592	0
Acidobacteria_Gp3	0.5497	0
Gallionella	0.5255	0
Na	0.5038	2.00E-07
Conexibacter	0.4897	4.00E-07
unclassified.Gammaproteobacteria	0.4760	9.00E-07
Roseiarcus	0.4667	1.60E-06
unclassified.Firmicutes	0.4641	1.90E-06
Telmatospirillum	0.4537	3.40E-06
unclassified.Rhodospirillaceae	0.4500	4.20E-06
Actinobacterium	0.4484	4.60E-06
Frankineae	0.4342	9.80E-06
Cl	0.4325	1.08E-05
Rhizomicrobium	0.4250	1.58E-05
Acidisphaera	0.4239	1.68E-05
Unclassified.Solirubrobacter	0.4050	4.24E-05
Actinomadura	0.3866	9.97E-05
unclassified.Betaproteobacteria	0.3851	0.000107
Acidobacteria_Gp1	0.3622	0.000287
Methylocystis	0.3499	0.000473
Sinobacteraceae	0.3485	0.000501
unclassified.Verrucomicrobia	0.3344	0.000866
Acidothermus	0.3256	0.001206
Thermomonosporaceae	0.3190	0.001532
Ca	0.3190	0.001534
Opitutus	0.3158	0.001719
Anaeromyxobacter	0.3113	0.002015
K	0.3068	0.00236
unclassified.Rubrobacteria	0.3061	0.002421
unclassified.Chlamydiales	0.3051	0.002499

unclassified.Opitutae	0.3024	0.002745
Rhodomicrobium	0.2993	0.003045
Granulibacter	0.2951	0.003503
Bdellovibrio	0.2879	0.004447
Porphyrobacter	0.2859	0.004742
stella	0.2855	0.004801
Kofleria	0.2831	0.005178
uncultured.Chloroflexi	0.2800	0.005716
unclassified.Myxococcales	0.2708	0.00761
Cryptosporangium	0.2705	0.007686
Thermoanaerobacteraceae	0.2681	0.008243
Spirochaeta	0.2609	0.010221
Prosthecobacter	0.2566	0.011585
Frankiaceae	0.2551	0.012123
Zavarzinella	0.2541	0.012451
unclassified.Acidimicrobiaceae	0.2477	0.014959
Actinospica	0.2471	0.015189
Wandonia	0.2415	0.017735
unclassified.Bauldia	0.2405	0.01823
Phaselicystis	0.2392	0.018872
Verrucomicrobium	0.2317	0.023067
Uncultured.Aquificae	0.2200	0.031191
Mucilaginibacter	0.2120	0.038056
Pseudolabrys	0.2092	0.040795
Aciditerrimonas	0.2081	0.041831
Terrimonas	-0.213	0.036319
Escherichia_Shigella	-0.216	0.034389
Amycolatopsis	-0.219	0.032045
Caulobacter	-0.230	0.023579
Burkholderia	-0.234	0.02152

					Pedobacter	-0.235	0.021154
					Leifsonia	-0.250	0.013934
					Gemmata	-0.251	0.013316
					stenotrophomonas	-0.259	0.010631
					Tardiphaga	-0.269	0.007944
					Beijerinckia	-0.275	0.006632
					Chitinophaga	-0.276	0.006372
					Mg	-0.278	0.005956
					Bacillus	-0.287	0.004537
					Hyphomicrobium	-0.318	0.001574
					streptomyces	-0.320	0.001434
					Afipia	-0.323	0.001327
					P	-0.355	0.000374
					TM7_genus_incertae_sedis	-0.358	0.000333
					Bosea	-0.360	0.000315
					Dyadobacter	-0.370	0.000199
					Sphingomonas	-0.380	0.000133
					Achromobacter	-0.382	0.000119
					Rhizobium	-0.386	0.000103
					Nocardioides	-0.391	8.02E-05
					Spirosoma	-0.391	7.87E-05
					Massilia	-0.403	4.64E-05
					Pullulanibacillus	-0.452	3.80E-06
					Cohnella	-0.458	2.60E-06
					Paenibacillus	-0.475	1.00E-06
					Methylobacterium	-0.491	4.00E-07
					Kaistia	-0.499	2.00E-07
					Pseudolabrys	0.6764	0
4	7.63%				*Armatimonadetes_gp5*	0.6690	0
		tpt2	0.279468	0.002697	*Ohtaekwangia*	0.6567	0

struvite	0.35278	0.003321	*Dongia*	0.6191	0
noplant	0.282319	0.04939	*Geothrix*	0.6151	0
sterile	0.176723	0.04939	*Spartobacteria*	0.6048	0
			Cytophaga	0.6015	0
			Thioalkalispiraceae	0.5899	0
			Schlesneria	0.5752	0
			Methylocystis	0.5730	0
			Hirschia	0.5727	0
			Devosia	0.5680	0
			Demequina	0.5575	0
			Asticcacaulis	0.5570	0
			Planctomyces	0.5546	0
			uncultured.Chloroflexi	0.5545	0
			unclassified.Bauldia	0.5373	0
			Spartobacteria_genera_incertae_sedis	0.5341	0
			Caulobacter	0.5319	0
			Archangium	0.5217	1.00E-07
			Bradyrhizobium	0.5200	1.00E-07
			Pasteuria	0.4923	3.00E-07
			Sediminibacterium	0.4891	4.00E-07
			Opitutus	0.4885	4.00E-07
			Kofleria	0.4844	6.00E-07
			Uncultured.Fibrobacter	0.4779	8.00E-07
			unclassified.Deltaproteobacteria	0.4779	8.00E-07
			unclassified.Rhizobiaceae	0.4703	1.30E-06
			Telmatospirillum	0.4679	1.50E-06
			Verrucomicrobiaceae	0.4551	3.20E-06
			Ferruginibacter	0.4474	4.90E-06
			Lysobacter	0.4140	2.75E-05
			Iamiaceae	0.3962	6.42E-05

Sphingobium	0.3959	6.52E-05
Parvibaculum	0.3876	9.54E-05
Na	0.3845	0.00011
Mesorhizobium	0.3833	0.000116
Rhodocyclaceae	0.3731	0.000181
unclassified.Halanobacteraceae	0.3685	0.00022
Flavobacterium	0.3656	0.000249
Rhodomicrobium	0.3635	0.000272
Rickettsia	0.3622	0.000287
uncultured.Planctomycetaceae	0.3572	0.000353
Uncultured.Pedobacter	0.3432	0.000618
Emticicia	0.3379	0.000758
Uncultured.Aquificae	0.3344	0.000865
Acidobacteria_Gp3	0.3321	0.000945
Novosphingobium	0.3143	0.001814
Terrimonas	0.3051	0.002504
Spirochaeta	0.2963	0.00337
NH4	0.2894	0.004238
Porphyrobacter	0.2873	0.004535
Polyangium	0.2856	0.004781
Unclassified.Sandaracinus	0.2730	0.007112
Sphingopyxis	0.2606	0.010333
Dyella	0.2564	0.011678
Phaselicystis	0.2519	0.013259
Luteolibacter	0.2517	0.013342
P	0.2492	0.014331
Peredibacter	0.2442	0.016479
unclassified.Betaproteobacteria	0.2423	0.017349
Escherichia_Shigella	0.2309	0.023583
Beijerinckia	0.2282	0.025279

Pseudonocardia	0.2165	0.034107
Mg	0.2123	0.037828
Legionella	0.2066	0.043381
Parachlamydia	0.2064	0.043559
Sphingobacterium	0.2033	0.046912
Uncultured.Pseudomonadaceae	0.2025	0.047832
unclassified.Myxococcales	-0.205	0.04473
Nocardia	-0.208	0.041888
Fluviicola	-0.215	0.034551
Anaeromyxobacter	-0.221	0.030233
Cryptosporangium	-0.223	0.028659
Conexibacter	-0.244	0.016381
Actinospica	-0.252	0.013071
unclassified.Acidimicrobiaceae	-0.258	0.01088
K	-0.260	0.010375
Wandonia	-0.261	0.010007
Burkholderia	-0.264	0.009216
Thermoanaerobacteraceae	-0.277	0.006194
Armatimonas_Armatimonadetes_gp1	-0.281	0.005461
stigmatella	-0.293	0.003675
Frankineae	-0.294	0.003544
Rhodoferax	-0.305	0.00251
Bdellovibrio	-0.353	0.000407
unclassified.Rubrobacteria	-0.355	0.000382
Roseiarcus	-0.372	0.000189
Acidobacteria_Gp14	-0.374	0.000171
Actinobacterium	-0.392	7.69E-05
Granulibacter	-0.398	5.82E-05
Acidobacteria_Gp1	-0.482	6.00E-07
Microbacterium	-0.482	6.00E-07

		Rhodanobacter	-0.533	0
		pH(H2O)	0.7736	0
		Armatimonas_Armatimonadetes_gp1	0.4650	1.80E-06
		Mucilaginibacter	0.4305	1.20E-05
		stigmatella	0.4020	4.90E-05
		unclassified.Roseateles	0.3497	0.000478
5	4.59%	*Na*	0.3245	0.001253
		unclassified.Bacteroidetes	0.2823	0.005322
		Wandonia	0.2772	0.006251
		Anaeromyxobacter	0.2711	0.007531
		NH4	0.2694	0.007945
		Peredibacter	0.2498	0.014103
		Ca	0.2246	0.027756
		Dyella	0.2121	0.037976
		Bosea	0.2116	0.038416
		Kaistia	0.2092	0.040731

Supplementary table 7.6: Correlations between relative bacterial abundances in the rhizosphere and plant, fertilizer, condition (sterile versus non-sterile) across time points, indicated by the Multiple Factor Analysis. Dimensions of the MFA can be described by the categorical variables included in the analysis. For each categorical variable (fertilizer, plant and time point), a one-way analysis of variance was performed with the coordinates of the samples on the axis, explained by either fertilizer, plant or time point. Then, for each level of the category (i.e. time point 1, time point 2 or time point 3 or tomato/lupine), a Hotelling T^2-test was used to compare the average of the category with the general average (using the constraint P i αi = 0, αi = 0). For instance, the coordinates of the relative abundance of genus "x" in tomato plants at time point 2 were compared with the average coordinates of the relative abundance of genus "x" in the tomato. The P value associated to this test is transformed to a normal quantile to assess whether the mean of the category is significantly less or greater than 0. Negative values indicate negative correlations.

Dimension	Variance	Descriptor	Estimate (R^2)	P value	Taxon	Correlation	*P* value
					Sphingomonas	0.786913	0
					Labrys	0.665789	0
					Kaistia	0.637228	1.00E-07
					Nocardioides	0.618279	3.00E-07
					Asticcacaulis	0.604649	6.00E-07
		nofert	1.155845	0.269167	*Verrucomicrobiaceae*	0.60275	7.00E-07
		lupine	0.339485	0.623453	*Rhizobium*	0.600362	8.00E-07
		tomato	-0.33949	0.623453	*Paenibacillus*	0.570449	3.60E-06
		organic	-0.85233	0.428614	*Roseomonas*	0.568548	4.00E-06
					Cupriavidus	0.568217	4.00E-06
1	13.29%				*Methylobacterium*	0.563025	5.10E-06
					Lysobacter	0.562202	5.30E-06
					Spirosoma	0.561775	5.40E-06
					Beijerinckia	0.558861	6.20E-06
					Peredibacter	0.548486	1.00E-05
					Tardiphaga	0.548252	1.01E-05
					Caulobacter	0.543782	1.23E-05
					Dyadobacter	0.525378	2.71E-05
					Pseudomonas	0.511431	4.79E-05
					Flavobacterium	0.470524	0.000221

Duganella	0.465664	0.000262
Uncultured.Clostridium	0.465664	0.000262
Candidatus.Paracaedibacteraceae	0.465664	0.000262
Leifsonia	0.463292	0.000284
Uncultured.Rhizobium	0.447184	0.000488
Hyphomicrobium	0.430213	0.000837
Pullulanibacillus	0.427246	0.000918
Archangium	0.415937	0.001292
Cohnella	0.406968	0.00168
Achromobacter	0.400777	0.002006
TM7_genus_incertae_sedis	0.394309	0.002405
Bacillus	0.394076	0.002421
Bosea	0.39222	0.002549
Escherichia_Shigella	0.376006	0.003946
Massilia	0.374201	0.004137
Chitinophaga	0.373556	0.004207
Gemmata	0.360134	0.005929
Niastella	0.357873	0.006272
Bradyrhizobium	0.357772	0.006288
Armatimonadetes_gp5	0.346927	0.008196
Demequina	0.346522	0.008276
Parvibaculum	0.34247	0.009116
Burkholderia	0.339244	0.009836
Cytophaga	0.333085	0.011349
Mesorhizobium	0.321278	0.014816
Rhodococcus	0.320769	0.014984
Uncultured.Fibrobacter	0.314309	0.017261
streptomyces	0.308958	0.019365
Xanthobacteraceae	0.305466	0.020852
Ohtaekwangia	0.298102	0.024308

Ramlibacter	0.297949	0.024384
Terrimonas	0.297363	0.024679
Byssovorax	0.297302	0.02471
Xanthomonadaceae	0.296573	0.025082
Dongia	0.293572	0.026664
Spartobacteria	0.291971	0.027541
Rhodocyclaceae	0.289928	0.028696
Collimonas	0.289759	0.028793
Luteibacter	0.2832	0.032789
Ferruginibacter	0.282878	0.032996
Rhodopirellula	0.282426	0.03329
Malawimonadaceae	0.274108	0.039082
Uncultured.Anaerolineae	0.273247	0.039727
Luteolibacter	0.270603	0.04176
unclassified.Halanobacteraceae	0.265107	0.046263
Uncultured.Pedobacter	0.264243	0.047006
Afipia	0.25797	0.052695
Rhodobacter	-0.26214	0.048852
Phenylobacterium	-0.28759	0.030066
Singulisphaera	-0.29355	0.026675
Acidisoma	-0.30591	0.020656
Rhizomicrobium	-0.3085	0.019555
Frankiaceae	-0.3114	0.018381
uncultured.Thermomicrobia	-0.34732	0.008119
Armatimonas_Armatimonadetes_gp1	-0.35567	0.006624
X3_genus_incertae_sedis	-0.35759	0.006317
unclassified.Roseateles	-0.3662	0.005087
stella	-0.3761	0.003935
unclassified.Firmicutes	-0.38039	0.003513
Wandonia	-0.38092	0.003464

Mucilaginibacter	-0.39345	0.002463
Acidisphaera	-0.39517	0.002348
Cryptosporangium	-0.41369	0.00138
Acidothermus	-0.41906	0.001177
Acidocella	-0.42977	0.000849
Thermoanaerobacteraceae	-0.43761	0.000664
unclassified	-0.44887	0.000462
Acidobacteria_Gp14	-0.45238	0.000411
unclassified.Actinomycetales	-0.45969	0.000322
unclassified.Myxococcales	-0.47737	0.000174
Rhodanobacter	-0.47932	0.000162
Actinospica	-0.4952	9.00E-05
Anaeromyxobacter	-0.50535	6.09E-05
Unclassified.Solirubrobacter	-0.51359	4.39E-05
unclassified.Rhodospirillaceae	-0.51443	4.25E-05
Rhodoferax	-0.54561	1.14E-05
unclassified.Gammaproteobacteria	-0.56554	4.60E-06
Methylovirgula	-0.57716	2.60E-06
unclassified.Rubrobacteria	-0.57721	2.60E-06
Bdellovibrio	-0.57743	2.60E-06
Sinobacteraceae	-0.58985	1.40E-06
Azospirillium	-0.59775	9.00E-07
Actinomadura	-0.60814	5.00E-07
Granulibacter	-0.67563	0
Conexibacter	-0.68981	0
unclassified.Acidimicrobiaceae	-0.72322	0
Frankineae	-0.74469	0
Roseiarcus	-0.77644	0
Actinobacterium	-0.79706	0
Acidobacteria_Gp1	-0.88247	0

					Pseudolabrys	0.736379	0
					Planctomyces	0.718345	0
					unclassified.Rhizobiaceae	0.706201	0
					Rhizomicrobium	0.705476	0
		tomato	2.767922	3.20E-06	*unclassified.Betaproteobacteria*	0.687155	0
		tpt2	2.330952	0.00094	*Ohtaekwangia*	0.684536	0
					Acidobacteria_Gp3	0.669538	0
					Armatimonadetes_gp5	0.660001	0
					Uncultured.Fibrobacter	0.649606	0
					uncultured.Chloroflexi	0.641603	1.00E-07
					Demequina	0.631907	1.00E-07
					Caulobacter	0.628411	2.00E-07
					Dongia	0.598781	9.00E-07
					Hirschia	0.594122	1.10E-06
					Geothrix	0.572036	3.30E-06
2	11.8%				*Terrimonas*	0.562014	5.40E-06
					Spartobacteria_genera_incertae_sedis	0.55669	6.90E-06
					Parvibaculum	0.546996	1.07E-05
					Thermomonosporaceae	0.546191	1.11E-05
					Parachlamydia	0.54301	1.28E-05
					Telmatospirillum	0.535206	1.79E-05
					Spartobacteria	0.532965	1.97E-05
					Archangium	0.530322	2.21E-05
					Asticcacaulis	0.526254	2.62E-05
					Opitutus	0.52398	2.88E-05
					Rhodocyclaceae	0.520462	3.32E-05
					uncultured.Planctomycetaceae	0.517435	3.76E-05
					Sphingobacterium	0.514709	4.20E-05
					unclassified.Bauldia	0.51331	4.44E-05
					Uncultured.Aquificae	0.506235	5.88E-05

Rhodopirellula	0.492483	9.97E-05
unclassified.Halanobacteraceae	0.492301	0.0001
Methylocystis	0.491776	0.000102
Gallionella	0.487977	0.000118
Sphingobium	0.486904	0.000123
Bradyrhizobium	0.486796	0.000123
Uncultured.Pedobacter	0.479974	0.000158
Ferruginibacter	0.479853	0.000159
Novosphingobium	0.472541	0.000206
Emticicia	0.463686	0.000281
unclassified.Deltaproteobacteria	0.461431	0.000303
Uncultured.Anaerolineae	0.458635	0.000333
Schlesneria	0.457848	0.000342
Kofleria	0.454164	0.000387
Gemmata	0.453124	0.000401
Larkinella	0.447207	0.000487
Iamiaceae	0.439839	0.000618
Xanthobacteraceae	0.435643	0.000707
Mesorhizobium	0.433224	0.000762
Luteolibacter	0.427262	0.000917
Byssovorax	0.422948	0.001046
Peredibacter	0.41885	0.001184
Pasteuria	0.410017	0.001538
Uncultured.Chlorobi	0.390812	0.00265
unclassified.Rhodospirillaceae	0.386875	0.002951
Gemmatimonas	0.378898	0.003655
Methylovirgula	0.350115	0.007589
Sediminibacterium	0.347087	0.008165
Thioalkalispiraceae	0.322202	0.014515
Prosthecobacter	0.308438	0.019581

Verrucomicrobiaceae	0.306455	0.020421
X3_genus_incertae_sedis	0.304428	0.021312
unclassified.Firmicutes	0.302487	0.022196
Lysobacter	0.28229	0.033378
Unclassified.Solirubrobacter	0.280369	0.034651
Devosia	0.276775	0.037141
Acidisphaera	0.272279	0.040461
Brevundimonas	0.271652	0.040944
Porphyrobacter	0.270856	0.041562
unclassified.Gammaproteobacteria	0.266622	0.044984
Azospirillium	0.265027	0.046331
Rhodobacter	0.263723	0.047457
unclassified.Bacteroidetes	0.263505	0.047648
Bacillus	-0.26404	0.04718
Nocardia	-0.26864	0.043327
Kribbella	-0.27461	0.038712
Cupriavidus	-0.29473	0.026044
Pseudomonas	-0.30172	0.022552
Bordetella	-0.3024	0.022238
Rhodococcus	-0.32976	0.012246
Massilia	-0.33136	0.011806
Malawimonadaceae	-0.33611	0.010583
Niastella	-0.358	0.006253
Afipia	-0.36946	0.004678
Microbacterium	-0.37504	0.004047
Rhodanobacter	-0.3871	0.002933
Kaistia	-0.38753	0.002899
Leifsonia	-0.39252	0.002527
Roseomonas	-0.39319	0.002481
Bosea	-0.40783	0.001638

3	5.35%			
		nofert	1.451575	0.024102

Burkholderia	-0.41602	0.001289
Pullulanibacillus	-0.41899	0.001179
Methylobacterium	-0.41907	0.001176
Achromobacter	-0.41921	0.001171
Paenibacillus	-0.42035	0.001132
streptomyces	-0.42527	0.000975
Chitinophaga	-0.45484	0.000379
Cohnella	-0.47507	0.000188
TM7_genus_incertae_sedis	-0.49651	8.56E-05
Nocardioides	-0.58187	2.10E-06
Duganella	0.710911	0
Uncultured.Clostridium	0.710911	0
Candidatus.Paracaedibacteraceae	0.710911	0
Dyadobacter	0.692729	0
Tardiphaga	0.655719	0
Spirosoma	0.622178	2.00E-07
Uncultured.Rhizobium	0.621137	3.00E-07
Pseudomonas	0.554037	7.80E-06
Kaistia	0.473398	0.0002
Cupriavidus	0.460437	0.000314
Adhaeribacter	0.432191	0.000787
Sphingomonas	0.4267	0.000933
Lysobacter	0.404242	0.001817
Bacillus	0.370151	0.004596
stigmatella	0.367447	0.004927
Beijerinckia	0.33895	0.009904
uncultured.Planctomycetaceae	0.314256	0.017281
Labrys	0.297621	0.024549
Rhizobium	0.289739	0.028805
Roseomonas	0.287021	0.030409

					Verrucomicrobiaceae	0.281805	0.033696
					Peredibacter	0.280003	0.034898
					Asticcacaulis	0.270607	0.041757
					Cytophaga	0.267809	0.044002
					Parvibaculum	-0.25737	0.053271
					Xanthomonadaceae	-0.26979	0.042404
					Larkinella	-0.27045	0.041884
					Pedobacter	-0.28272	0.033096
					Paenibacillus	-0.2846	0.031902
					Armatimonadetes_gp5	-0.28874	0.029386
					Escherichia_Shigella	-0.2891	0.029176
					Demequina	-0.29222	0.027404
					Rhodanobacter	-0.29611	0.025324
					Afipia	-0.30662	0.020349
					Legionella	-0.31693	0.016303
					Burkholderia	-0.32572	0.013419
					Dokdonella	-0.34885	0.007824
					Niastella	-0.40181	0.001948
					Rhodococcus	-0.42247	0.001062
					Malawimonadaceae	-0.47088	0.000219
					Kribbella	-0.47106	0.000217
					Cohnella	-0.5064	5.84E-05
					streptomyces	-0.50886	5.30E-05
					Chitinophaga	-0.51258	4.57E-05
4	5.14%	lupine	1.501756	0.000207	*Larkinella*	0.597984	9.00E-07
					Terrimonas	0.531318	2.11E-05
					Pedobacter	0.529239	2.31E-05
					Xanthobacteraceae	0.523629	2.92E-05
		tpt2	1.37503	0.003327	*Geothrix*	0.493105	9.74E-05
					Emticicia	0.488516	0.000116

Sphingobacterium	0.480601	0.000155
Verrucomicrobiaceae	0.476939	0.000176
Armatimonadetes_gp5	0.470074	0.000225
Aciditerrimonas	0.458606	0.000334
Demequina	0.433874	0.000747
Hirschia	0.424061	0.001012
Ohtaekwangia	0.382934	0.003282
Conexibacter	0.364946	0.005251
unclassified.Acidimicrobiaceae	0.360982	0.005804
Parachlamydia	0.321768	0.014656
Actinobacterium	0.316204	0.016565
Acidisoma	0.316032	0.016627
Spirochaeta	0.303347	0.021801
Uncultured.Fibrobacter	0.298257	0.02423
unclassified.Roseateles	0.294953	0.025926
Thermomonosporaceae	0.294336	0.026254
Armatimonas_Armatimonadetes_gp1	0.289904	0.02871
Spirosoma	0.280267	0.034719
Unclassified.Solirubrobacter	0.280098	0.034833
Acidobacteria_Gp3	-0.25706	0.053569
Collimonas	-0.26097	0.049905
Nocardia	-0.26364	0.047526
Dongia	-0.27329	0.039695
Flavisolibacter	-0.27614	0.037593
unclassified.Firmicutes	-0.28394	0.032319
Rhodobacter	-0.29738	0.024671
Alkanibacter	-0.30073	0.02302
Novosphingobium	-0.31648	0.016466
Mycobacterium	-0.31729	0.016178
Rhizomicrobium	-0.33388	0.011143

					Sphaerobacter	-0.36205	0.00565
					Hyphomicrobium	-0.38505	0.0031
					Devosia	-0.38656	0.002976
					Afipia	-0.39209	0.002558
					Kofleria	-0.41349	0.001389
					Mesorhizobium	-0.42604	0.000952
					Porphyrobacter	-0.43077	0.000823
					Nitrosospira	-0.48377	0.000138
					Gemmatimonas	-0.56521	4.60E-06
					OD1_genus_incertae_sedis	-0.57203	3.30E-06
					Uncultured.Anaerolineae	0.625177	2.00E-07
					Luteolibacter	0.595907	1.00E-06
					Rhodopirellula	0.57996	2.30E-06
		tpt2	1.424805	0.001295	*Byssovorax*	0.57147	3.40E-06
		lupine	1.053399	0.008034	*Sediminibacterium*	0.499882	7.52E-05
					Uncultured.Chlorobi	0.480715	0.000154
5	4.67%				*Armatimonas_Armatimonadetes_gp1*	0.444461	0.000533
					Spartobacteria	0.44182	0.00058
					Thioalkalispiraceae	0.436721	0.000683
					Sorangiineae	0.398132	0.002161
					Singulisphaera	0.392651	0.002518
					Uncultured.Pedobacter	0.363756	0.005412
					Archangium	0.363549	0.00544
					Dokdonella	0.307716	0.019883
					unclassified	0.302048	0.0224
					Mucilaginibacter	0.296879	0.024926
					Pasteuria	0.289187	0.029125
					Methylovirgula	0.282084	0.033513
					unclassified.Bauldia	0.276395	0.037412
					Acidisoma	0.27448	0.038806

Parvibaculum	0.272408	0.040363
Lysobacter	0.270718	0.04167
Iamiaceae	0.269815	0.042383
Ferruginibacter	0.268003	0.043843
Prosthecobacter	0.261151	0.049743
unclassified.Myxococcales	-0.26296	0.048126
Adhaeribacter	-0.26406	0.047165
X3_genus_incertae_sedis	-0.26987	0.042341
Porphyrobacter	-0.27657	0.037287
Parachlamydia	-0.27678	0.037135
Zavarzinella	-0.27761	0.036548
uncultured.Planctomycetaceae	-0.29092	0.028129
Devosia	-0.29549	0.025641
Hirschia	-0.29589	0.025436
Collimonas	-0.29877	0.023975
Emticicia	-0.29879	0.023964
uncultured.Chloroflexi	-0.31042	0.018771
unclassified.Opitutae	-0.31339	0.017608
Uncultured.Aquificae	-0.35687	0.00643
Rhodobacter	-0.36089	0.005818
Telmatospirillum	-0.37004	0.004609
Xanthobacteraceae	-0.38859	0.002816
Terrimonas	-0.39519	0.002347
Larkinella	-0.43085	0.000821
Sphingobacterium	-0.43144	0.000806
Geothrix	-0.44811	0.000473
Gemmata	-0.4772	0.000175
Gemmatimonas	-0.5109	4.89E-05

Supplementary table 7.7: Effect of plant and fertilizer on bacterial relative abundance detected at the final harvest (time point 3, n = 12) in root rhizosphere. Analysis was performed separately for root and bulk zone to consider time effect and to uncover whether the final bacterial relative abundance was impacted only by either fertilizer or plant type and not by time. The genera included in this table are only those whose relative abundance was significantly different at the end of the experiment as a result of fertilizer or plant effect. NF, no fertilizer; ORG, Organic fertilizer; STR, struvite. NS= not significantly different.

Taxon	Fertilizer	Plant		Effect		
		Lupine	Tomato	Plant	Fertilizer	Plant*Fertilizer
Acidisphaera	NF	0.1431 ± 0.009	0.2545 ± 0.0569	0.03	NS	NS
	ORG	0.1508 ± 0.080	0.1834 ± 0.0260			
	STR	0.1270 ± 0.0115	0.2365 ± 0.0618			
Aciditerrimonas	NF	0.0049 ± 0.0012	0.0037 ± 0.0028	NS	0.004	NS
	ORG	0.0032 ± 0.0009	0.0035 ± 0.002			
	STR	0.0116 ± 0.0006	0.0148 ± 0.0057			
Acidobacteria Gp3	NF	0.0561 ± 0.020	0.3991 ± 0.0412	<0.0001	NS	NS
	ORG	0.1064 ± 0.0123	0.1758 ± 0.0432			
	STR	0.0826 ± 0.0606	0.2682 ± 0.0391			
Acidothermus	NF	0.0179 ± 0.0008	0.0186 ± 0.0039	NS	0.03	NS
	ORG	0.0131 ± 0.0033	0.0195 ± 0.0088			
	STR	0.0197 ± 0.0102	0.0436 ± 0.0107			
Aquicella	NF	0.0016 ± 0.0016	0.0043 ± 0.0023	NS	0.03	NS
	ORG	0.0026 ± 0.002	0.0029 ± 0.0012			
	STR	0.0051 ± 0.0045	0.0119 ± 0.0043			
Azospirillum	NF	0.2540 ± 0.055	0.5247 ± 0.2736	0.04	NS	NS
	ORG	0.1759 ± 0.069	0.2960 ± 0.0827			
	STR	0.1786 ± 0.0759	0.2958 ± 0.065			
Byssovorax	NF	0.0011 ± 0.0014	0.0017± 0.0013	0.009	NS	NS
	ORG	0.0000±0.0005	0.0038 ± 0.0028			
	STR	0.0007 ± 0.0005	0.0031 ± 0.0015			

Caedibacter	NF	0.0020 ± 0.001	0.0009 ± 0.0007	0.002	NS	NS
	ORG	0.0002 ± 0.0002	0.0022 ± 0.0025			
	STR	0.0004 ± 0.0003	0.0021 ± 0.0015			
Cellulomonas	NF	0.0002 ± 0.0002	0.0002 ± 0.0002	NS	0.001	NS
	ORG	0.0004 ± 0.0003	0.0002 ± 0.0001			
	STR	0.0010 ± 0.001	0.0010 ± 0.0014			
Escherichia Shigella	NF	0.0384 ± 0.0101	0.0501 ± 0.0258	0.03	NS	NS
	ORG	0.0749 ± 0.0431	0.0584 ± 0.011			
	STR	0.0177 ± 0.0113	0.0463 ± 0.0196			
Flavisolibacter	NF	0.0005 ± 0.0005	0.0029 ± 0.0011	<0.0001	NS	NS
	ORG	0.0002 ± 0.0001	0.0035 ± 0.0034			
	STR	0.0003 ± 0.0002	0.0034 ± 0.0035			
Frankiaceae	NF	0.0119 ± 0.0027	0.0272 ± 0.0144	0.03	NS	NS
	ORG	0.0137 ± 0.001	0.0294 ± 0.013			
	STR	0.0177 ± 0.0107	0.0510 ± 0.0255			
Gallionella	NF	0.0097 ± 0.0097	0.0645 ± 0.0147	0.003	NS	NS
	ORG	0.0052 ± 0.0016	0.0135 ± 0.0086			
	STR	0.0109 ± 0.0128	0.0245 ± 0.010			
Gemmatimonas	NF	0.0824 ± 0.0159	0.6621 ± 0.1342	<0.0001	NS	NS
	ORG	0.1008 ± 0.0022	0.3565 ± 0.067			
	STR	0.0976 ± 0.0549	0.4371± 0.1019			
Granulibacter	NF	0.4236 ± 0.0355	0.9115 ± 0.5563	0.04	NS	NS
	ORG	0.4221 ± 0.0817	0.5891 ± 0.0961			
	STR	0.2775 ± 0.0752	0.6594 ± 0.0673			
Hypomicrobium	NF	0.0338 ± 0.0049	0.0758 ± 0.0112	<0.0001	NS	NS
	ORG	0.0187 ± 0.0057	0.0573 ± 0.0124			
	STR	0.0410 ± 0.0258	0.0553 ± 0.0179			
Kofleria	NF	0.0073 ± 0.0035	0.1027 ± 0.0243	<0.0001	NS	NS
	ORG	0.0038 ± 0.0029	0.0351 ± 0.0187			

	STR	0.0048 ± 0.0035	0.0299 ± 0.0136			
	NF	0.0932 ± 0.0112	0.2885 ± 0.0289			
Mesorhizobium	ORG	0.1223 ± 0.0102	0.3563 ± 0.0952	<0.0001	NS	NS
	STR	0.1500 ± 0.0156	0.4300 ± 0.0696			
	NF	0.0695 ± 0.0079	0.1104 ± 0.0318			
Methylocystis	ORG	0.0492 ± 0.002	0.0972 ± 0.0237	<0.0001	NS	NS
	STR	0.0380 ± 0.0193	0.1995 ± 0.1044			
	NF	0.0027 ± 0.0027	0.0287 ± 0.0073			
Nitrosospira	ORG	0.0033 ± 0.0026	0.0341 ± 0.0131	0.04	NS	NS
	STR	0.0028 ± 0.0022	0.0180 ± 0.0057			
	NF	0.0250 ± 0.0182	0.1126 ± 0.0215			
Novosphingobium	ORG	0.0183 ± 0.0117	0.0749 ± 0.023	0.0006	NS	NS
	STR	0.0409 ± 0.033	0.0842 ± 0.0174			
	NF	0.0059 ± 0.0059	0.0212 ± 0.0057			
Parachlamydia	ORG	0.0054 ± 0.0055	0.0119 ± 0.0076	0.007	NS	NS
	STR	0.0066 ± 0.005	0.0280 ± 0.0157			
	NF	0.0001 ± 0.0001	0.0017 ± 0.0007			
Phaselicystis	ORG	0.0001 ± 0.0001	0.0027 ± 0.0015	<0.0001	NS	NS
	STR	0.0003 ± 0.0002	0.0044 ± 0.0045			
	NF	0.0077 ± 0.0039	0.0084 ± 0.006			
Polyangium	ORG	0.0010 ± 0.0008	0.0010 ± 0.0006	NS	0.006	NS
	STR	0.0131 ± 0.0105	0.0052 ± 0.0029			
	NF	0.0279 ± 0.0223	0.1529 ± 0.0228			
Porphyrobacter	ORG	0.0385 ± 0.0061	0.1552 ± 0.0071	<0.0001	NS	NS
	STR	0.0661 ± 0.0366	0.2271 ± 0.0538			
	NF	0.0001 ± 0.0001	0.0001 ± 0.0001			
Propionibacterium	ORG	0.0001 ± 0.00001	0.0007 ± 0.0007	NS	0.04	NS
	STR	0.00001 ±0.00001	0.0001 ± 0.0001			
Rhizomicrobium	NF	2.2817 ± 0.0231	6.6243 ± 0.5650	<0.0001	NS	NS

	ORG	3.0220 ± 0.4682	4.2094 ± 0.2378			
	STR	2.4236 ± 0.3406	5.1653 ± 0.8295			
	NF	0.0076 ± 0.0077	0.0745 ± 0.0215			
Rhodobacter	ORG	0.0158 ± 0.0025	0.0540 ± 0.0117	0.0006	NS	NS
	STR	0.0104 ± 0.0084	0.0609 ± 0.0168			
	NF	0.0927 ± 0.0123	0.3479 ± 0.034			
Rhodoferax	ORG	0.2189 ± 0.0765	0.3072 ± 0.0429	0.0007	NS	NS
	STR	0.0972 ± 0.0918	0.4022 ± 0.075			
	NF	0.0005 ± 0.0005	0.0041 ± 0.0017			
Rhodomicrobium	ORG	0.0002 ± 0.0001	0.0021 ± 0.0021	<0.0001	NS	NS
	STR	0.0004 ± 0.0003	0.0044 ± 0.0037			
	NF	0.0171 ± 0.0033	0.0421± 0.014			
Stella	ORG	0.0100 ± 0.0044	0.0164 ± 0.0053	0.04	NS	NS
	STR	0.0194 ± 0.0088	0.0324 ± 0.0037			
	NF	0.0066 ± 0.007	0.0409 ± 0.0153			
Telmatospirillum	ORG	0.0128 ± 0.0032	0.0358 ± 0.0073	0.003	NS	NS
	STR	0.0097 ± 0.0026	0.0346 ± 0.0144			
	NF	0.0168 ± 0.0035	0.0766 ± 0.0083			
Thermoanaerobacteraceae	ORG	0.0250 ± 0.016	0.0431 ± 0.0162	0.005	NS	NS
	STR	0.0163 ± 0.0119	0.0487 ± 0.0122			
	NF	0.0813 ± 0.0079	0.1083 ± 0.0194			
Thermomonosporaceae	ORG	0.0811 ± 0.037	0.1051 ± 0.0224	0.006	NS	NS
	STR	0.0617 ± 0.0418	0.1698± 0.0460			
	NF	0.0095 ± 0.0095	0.0650± 0.0063			
Unclassified *Betaproteobacteria*	ORG	0.0090 ± 0.0049	0.0432± 0.0047	<0.0001	NS	NS
	STR	0.0094 ± 0.0042	0.0457± 0.0101			
	NF	0.0007 ± 0.0007	0.0023 ± 0.0018			
Unclassified *Chloroflexi*	ORG	0.0013 ± 0.001	0.0079 ± 0.0079	0.002	NS	NS
	STR	0.0011 ± 0.0008	0.0047 ± 0.0041			

Unclassified *Deltaproteobacteria*	NF	0.0140 ± 0.0071	0.0127 ± 0.0052	0.001	NS	NS
	ORG	0.0041 ± 0.0032	0.0155 ± 0.0108			
	STR	0.0067 ± 0.0021	0.0186 ± 0.0074			
Unclassified *Firmicutes*	NF	0.0123 ± 0.0035	0.0411 ± 0.0027	0.003	NS	NS
	ORG	0.0099 ± 0.0071	0.0494 ± 0.0103			
	STR	0.0204 ± 0.0150	0.0425 ± 0.012			
Unclassified *Gammaproteobacteria*	NF	0.1421 ± 0.0193	0.4084 ± 0.1631	0.002	NS	NS
	ORG	0.1622 ± 0.031	0.2830 ± 0.0428			
	STR	0.1900 ± 0.1081	0.2732 ± 0.0288			
Unclassified *Halanobacteraceae*	NF	0.0005 ± 0.0005	0.0038 ± 0.0017	<0.0001	NS	NS
	ORG	0.0004 ± 0.0003	0.0033 ± 0.0018			
	STR	0.0007 ± 0.0005	0.0055 ± 0.0057			
Unclassified *Opitutaceae*	NF	0.0051 ± 0.0051	0.0273 ± 0.0052	0.02	NS	NS
	ORG	0.0071 ± 0.0023	0.0198 ± 0.0071			
	STR	0.0084 ± 0.0120	0.0186 ± 0.0075			
Unclassified *Rhodospirillaceae*	NF	0.0807 ± 0.0058	0.2251 ± 0.0538	0.006	NS	NS
	ORG	0.1140 ± 0.0467	0.1937 ± 0.0297			
	STR	0.0880 ± 0.0556	0.2667 ± 0.0703			
Unclassified *Roseateles*	NF	0.0040 ± 0.0017	0.0019 ± 0.0013	0.03	NS	NS
	ORG	0.0020 ± 0.0019	0.0007 ± 0.0004			
	STR	0.0047 ± 0.0011	0.0025 ± 0.0014			
Unclassified *Sandaracinus*	NF	0.0005 ± 0.0005	0.0009 ± 0.0007	0.008	NS	NS
	ORG	0.0003 ± 0.0002	0.0040 ± 0.0037			
	STR	0.0003 ± 0.0002	0.0019 ± 0.0014			
Unclassified Sphingobacter	NF	0.0148 ± 0.0149	0.0379 ± 0.0070	NS	0.02	NS
	ORG	0.0177 ± 0.0084	0.0405 ± 0.0130			
	STR	0.0629 ± 0.0369	0.0577 ± 0.0105			
Uncultured *Aquificae*	NF	0.0025 ± 0.0025	0.0191 ± 0.0039	<0.0001	NS	NS
	ORG	0.0016 ± 0.0012	0.0203 ± 0.0126			

	STR	0.0021 ± 0.0016	0.0186 ± 0.0121			
	NF	0.0050 ± 0.0056	0.0329 ± 0.0050			
Uncultured *Chloroflexi*	ORG	0.0049 ± 0.0045	0.0216 ± 0.0037	<0.0001	NS	NS
	STR	0.0074 ± 0.0091	0.0414 ± 0.0139			
	NF	0.0015 ± 0.0015	0.0078 ± 0.0023			
Uncultured *Pedobacter*	ORG	0.0021 ± 0.0016	0.0122 ± 0.0056	0.0004	NS	NS
	STR	0.0020 ± 0.0015	0.0107 ± 0.0056			
	NF	0.0329 ± 0.0003	0.0749 ± 0.0320			
Verrucomicrobium	ORG	0.0414 ± 0.0056	0.0871 ± 0.0289	0.006	NS	NS
	STR	0.0331 ± 0.0086	0.0790 ± 0.0412			
	NF	0.0006 ± 0.0006	0.0122 ± 0.0058			
Zavarzinella	ORG	0.0004 ± 0.0003	0.0060 ± 0.0062	<0.0001	NS	NS
	STR	0.0005 ± 0.0004	0.0063 ± 0.0034			

Supplementary table 7.8: Effect of plant and fertilizer on bacterial relative abundance detected at the final harvest (time point 3, n = 12) in bulk zone. Analysis was performed separately for root and bulk zone to consider time effect and to uncover whether the final bacterial relative abundance was impacted only by either fertilizer or plant type and not by time. The genera included in this table are only those whose relative abundance was significantly different at the end of the experiment as a result of fertilizer or plant effect. NF, no fertilizer; OR, organic fertilizer; STR, struvite. NS= not significantly different.

Taxon	Fertilizer	Plant		Effect		
		Lupine	Tomato	Plant	Fertilizer	Plant*Fertilizer
3X genus Incertae sedis	NF	0.4671 ± 0.1583	0.5255 ± 0.1257	0.02	NS	0.0006
	ORG	0.1393 ± 0.0288	0.6995 ± 0.0721			
	STR	0.4281 ± 0.0257	0.3903 ± 0.0842			
Acidisphaera	NF	0.2197 ± 0.0651	0.2610 ± 0.0381	NS	0.05	<0.0001
	ORG	0.0898 ± 0.0099	0.1619 ± 0.0628			
	STR	0.1736 ± 0.0114	0.1264 ± 0.0322			
Aciditerrimonas	NF	0.0035 ± 0.0009	0.0077 ± 0.0025	NS	0.02	0.01
	ORG	0.0041 ± 0.0012	0.0098 ± 0.0064			
	STR	0.0097 ± 0.0022	0.0088 ± 0.0039			
Aquicella	NF	0.0111 ± 0.0106	0.0043 ± 0.0022	0.01	NS	NS
	ORG	0.0025 ± 0.0025	0.0026 ± 0.0010			
	STR	0.0155 ± 0.0084	0.0042 ± 0.0020			
Armatimonas Armatimonadetes gp1	NF	0.0679 ± 0.0223	0.0196 ± 0.0071	0.01	NS	0.03
	ORG	0.0126 ± 0.0091	0.0130 ± 0.0079			
	STR	0.0293 ± 0.0095	0.0144 ± 0.0045			
Asticcacaulis	NF	0.3267 ± 0.0902	0.1412 ± 0.0463	<0.0001	NS	<0.0001
	ORG	0.0074 ± 0.0000	0.2644 ± 0.0191			
	STR	0.1005 ± 0.0003	0.2795 ± 0.1093			
Brucella	NF	0.0004 ± 0.0003	0.0005 ± 0.0004	NS	<0.0001	NS
	ORG	0.0009 ± 0.0009	0.0017 ± 0.0011			
	STR	0.0011 ± 0.0015	0.0010 ± 0.0005			

Caedibacter	NF	0.0004 ± 0.0003	0.0005 ± 0.0003	0.01	NS	NS
	ORG	0.0005 ± 0.0005	0.0010 ± 0.0006			
	STR	0.0010 ± 0.0025	0.0005 ± 0.0003			
Dongia	NF	0.0204 ± 0.0101	0.0440 ± 0.0071	0.0001	NS	NS
	ORG	0.0102 ± 0.0073	0.0448 ± 0.0099			
	STR	0.0191 ± 0.0094	0.0304 ± 0.0062			
Gallionella	NF	0.0014 ± 0.0011	0.0052 ± 0.0022	0.0002	NS	NS
	ORG	0.0031 ± 0.0031	0.0328 ± 0.0179			
	STR	0.0037 ± 0.0001	0.0087 ± 0.0059			
Hirschia	NF	0.0030 ± 0.0073	0.0055 ± 0.0017	0.002	NS	NS
	ORG	0.0017 ± 0.0017	0.0077 ± 0.0028			
	STR	0.0044 ± 0.0018	0.0062 ± 0.0030			
Lysobacter	NF	0.0087 ± 0.0066	0.0335 ± 0.0110	<0.0001	NS	NS
	ORG	0.0119 ± 0.0075	0.1081 ± 0.0795			
	STR	0.0493 ± 0.0234	0.0403 ± 0.0105			
Paenibacillus	NF	0.0682 ± 0.0211	0.0283 ± 0.0116	0.05	NS	NS
	ORG	0.1356 ± 0.0316	0.0374 ± 0.0167			
	STR	0.0403 ± 0.0265	0.0183 ± 0.0069			
Parachlamydia	NF	0.0347 ± 0.0050	0.0200 ±0.0031	0.03	NS	NS
	ORG	0.0155± 0.0081	0.0195 ± 0.0081			
	STR	0.0382 ± 0.015	0.0153± 0.0069			
Parvibaculum	NF	0.0030 ± 0.0023	0.0131± 0.0072	0.04	NS	NS
	ORG	0.0033 ± 0.0033	0.0095± 0.0037			
	STR	0.0064 ± 0.0053	0.0074± 0.0027			
Spartobacteria	NF	0.0130 ± 0.0100	0.0097± 0.0050	0.003	NS	NS
	ORG	0.0019 ± 0.0019	0.0083± 0.0036			
	STR	0.0042 ± 0.0042	0.0056± 0.0015			
Sphingobacterium	NF	0.0020 ± 0.0015	0.0078± 0.0024	<0.0001	NS	NS
	ORG	0.0034 ± 0.0034	0.0324± 0.0224			

	STR	0.0036 ± 0.0005	0.0080± 0.0022			
	NF	0.0129 ± 0.0099	0.0254± 0.0117			
Sphingobium	ORG	0.0149 ± 0.0015	0.0288± 0.0098	0.03	NS	NS
	STR	0.0218 ± 0.0115	0.0141± 0.0043			
	NF	0.0005 ± 0.0004	0.0004± 0.0003			
Stenotrophomonas	ORG	0.0470 ± 0.0030	0.0012± 0.0008	NS	0.0008	NS
	STR	0.0021 ± 0.0021	0.0006± 0.0003			
	NF	0.0070 ± 0.0092	0.0117± 0.0041			
Telmatospirillum	ORG	0.0044 ± 0.0044	0.0352± 0.0084	0.0006	NS	NS
	STR	0.0077 ± 0.0042	0.0144± 0.0042			
	NF	0.3402 ± 0.0513	0.1947± 0.0204			
Unclassified	ORG	0.1469 ± 0.0107	0.2432± 0.1164	0.05	NS	NS
	STR	0.1586 ± 0.0212	0.1082± 0.0084			
	NF	0.0008 ± 0.0006	0.0081± 0.0043			
Unclassified *Bauldia*	ORG	0.0006 ± 0.0006	0.0085± 0.0015	<0.0001	NS	NS
	STR	0.0017 ± 0.0017	0.0087± 0.0058			
	NF	0.0040 ± 0.0031	0.0128± 0.0029			
Unclassified *Opitutae*	ORG	0.0091 ± 0.0091	0.0446± 0.0333	0.03	NS	NS
	STR	0.0139 ± 0.0019	0.0187± 0.0087			
	NF	0.0032 ± 0.0040	0.0020± 0.0013			
Unclassified *Roseateles*	ORG	0.0010 ± 0.0010	0.0011± 0.0007	0.002	NS	NS
	STR	0.0018 ± 0.0018	0.0014± 0.0012			
	NF	0.0711 ± 0.0192	0.0571± 0.0220			
Unclassified *Verrucomicrobia*	ORG	0.0319 ± 0.0287	0.0592± 0.0098	0.006	NS	NS
	STR	0.0475 ± 0.0444	0.0357± 0.0225			
	NF	0.0003 ± 0.0003	0.0030± 0.0051			
Uncultured *Anaerolineae*	ORG	0.0001 ± 0.0001	0.0008± 0.0005	<0.0001	NS	NS
	STR	0.0003 ± 0.0003	0.0013± 0.0006			
Uncultured *Pedobacter*	NF	0.0015 ± 0.0012	0.0076± 0.0057	0.0006	NS	NS

	ORG	0.0019 ± 0.0019	0.0133± 0.0163			
	STR	0.0026 ± 0.0026	0.0061± 0.0018			
	NF	0.0247 ± 0.0246	0.0036± 0.0024			
Uncultured *Rhizobium*	ORG	0.0187 ± 0.0025	0.0111± 0.0073	0.05	NS	NS
	STR	0.0047 ± 0.0047	0.0024± 0.0012			
	NF	0.0019 ± 0.0024	0.0046± 0.0034			
Verrucomicrobiaceae	ORG	0.0018 ± 0.0012	0.0064± 0.0027	0.02	NS	NS
	STR	0.0020 ± 0.0020	0.0036± 0.0047			
	NF	0.0658 ± 0.0351	0.0344± 0.0085			
Verrucomicrobium	ORG	0.0414 ± 0.0104	0.0459± 0.0190	NS	0.03	NS
	STR	0.0796 ± 0.0103	0.0544± 0.0182			

*Supplementary table 7.9: Correlations (r) among chemical characteristics and plant performance (leaf area, fresh weight, dry weight and dry matter content) in organic growing medium for cultivating tomato plants, with struvite fertilizer (n = 5). In parenthesis, the time point when the correlation was observed. ***P < 0.0001, **P < 0.05*

	Conductivity	P	K	SO_4^{2-}	Na	Cl	Leaf area	Fresh weight	Dry weight	Dry matter content (%)
pH(H2O)	-0.969** (2)									
Conductivity			0.908** (3)		-0.879** (3)	0.962**(3)				0.926** (2)
NH_4^+-N		0.988** (2)								
P			-0.929** (3)			-0.901** (3)	0.992*** (2) 0.978**(3)	0.918**(2)	0.901**(2)	0.946** (3)
K				0.951** (3)		0.967** (3)	-0.912** (3)			-0.923** (3)
Leaf area								0.937** (2)	0.927** (2)	
Fresh weight									0.970** (2)	

*Supplementary table 7.10: Correlations (r) among chemical characteristics and plant performance (leaf area, fresh weight, dry weight and dry matter content in organic growing medium for cultivating tomato plants, with organic fertilizer (n = 5). In parenthesis, the time point when the correlation was observed. ***P < 0.0001, **P < 0.05.*

	Mg	**SO_4^{2-}**	**Cl**	**Fresh weight**	**Dry weight**
Conductivity		0.964*** (3)	0.948** (2) 0.979** (3)		
Ca	0.881** (3)				
Cl				-0.889** (2)	
Fresh weight					0.948**(2)

*Supplementary table 7.11: Correlations (r) among chemical characteristics and plant performance (leaf area, fresh weight, dry weight and dry matter content) in organic growing medium for cultivating tomato plants, without fertilizer (n = 5). In parenthesis, the time point when the correlation was observed. ***P < 0.0001, **P < 0.05.*

	Conductivity	**P**	**K**	**SO_4^{2-}**	**Cl**	**Leaf area**	**Fresh weight**	**Dry weight**
pH(H2O)	-0.982** (2)			-0.993*** (2)	-0.980** (3)			
Conductivity			0.977**(3)	0.903** (3)	0.999*** 2)			
NH_4^+-N		0.881** (3)						
P			-0.913** (2)					
Ca						-0.916** (3)		
SO_4^{2-}					0.975** (2)			
Leaf area							0.974** (2) 0.915** (3)	0.915**(2)
Fresh weight								0.995***(3)

CHAPTER 8: GENERAL DISCUSSION

1. General discussion

This chapter presents a general discussion of the results obtained during this PhD, as well as some perspectives for future research. The main aims of this doctoral study was i) to study the microbial community composition associated with the growing medium, ii) to quantify key functionalities (respiration, ammonia and nitrite oxidation rate) of individual growing medium constituents, iii) to link the N dynamics with the microbial community associated with the growing medium and the plant and iv) to setup some proof of concepts concerning fertigation strategies with organic fertilizers

As this thesis is done in close cooperation with the growing media industry, it was also expected to:

- define critical parameters with respect to internal quality control of these growing media
- find solutions for the high ammonium/ammonia concentrations in growing media blended with organic fertilizer resulting in too high pH values
- develop novel soilless culture systems for the production of vegetables in combination with the predefined organic growing medium (GB), which was used throughout all the tests.

2. Soilless culture microbial communities

H1: The organic growing medium (GB) has a higher species diversity compared to a mineral growing medium (RW)

RQ1: 'Do organic growing media have a higher species richness, diversity, and evenness compared to mineral growing media in closed soilless culture systems?

Chapter 2 showed that the soilless microbial community structure in combination with organic growing media was distinctly different, i.e. higher species richness, evenness and diversity, compared to mineral growing media.

Microbial ecology aims at understanding microorganisms in the environment and their interactions with each other. The rapid accumulation of molecular data is uncovering abundant uncultivated microbial groups and novel microbial functions (Prosser et al. 2007). Microbial diversity in soil ecosystems exceeds, by far, that of eukaryotic organisms and encloses genetic variability within species, the species richness and relative abundance (evenness) of taxa and functional groups in communities.

Soilless culture systems and the growing media used in horticulture differ in terms of physical and chemical properties and consequently it is very likely that their microbial community composition and structures are different too (Khalil and Alsanius 2001; Koohakan et al. 2004). Research showed that mineral growing media were mainly colonized by bacteria, while the organic growing media had a larger fungal community mainly as a result of the type of organic compounds available to the microorganisms (Koohakan et al. 2004), however little is known about soilless community composition and structure. Grayston et al. (2001) indicates that the type and amount of available organic nutrients strongly influence the abundance of microbial groups and their functional diversity in soil ecosystems. Smit et al. (2001) suggested that soil with a high content of readily available nutrients showed positive selection for α- and γ-proteobacteria, this being indicative of r-selection, which is selection for bacteria with potentially high growth rates. In our study (Chapter 2), *Rhodocyclaceae* and *Methylophilaceae* (β-

Proteobacteria), were correlated to the organic growing medium GB, as well as other α-, β and ϒ-Proteobacteria, such as *Hyphomicrobiaceae*, *Xanthobacteraceae*, *Phyllobacteriaceae*, and *Chromatiaceae*. Actinobacteria such as *Gaiellaceae* and *Conexibacteraceae* were also positively correlated with GB. In low-nutrient soils, the relative abundance of *Acidobacterium* is indicative of k-selection, which is selection for bacteria with lower growth potential but higher capability to compete for nutrients. In our study, the relative abundance of *Acidobacterium* was also positively correlated to GB (Supplementary table 2.3), indicating a selection of bacteria with a high capability of nutrient competition.

Previous research dealing with the relationship between biodiversity and functioning has focused mainly on species richness. Bacterial species richness and ecosystem functionality and stability are positively correlated (Bell et al., 2005). The interconnectedness between evenness and functioning, has received less attention. It was demonstrated by Wittebolle (2009) that – even for communities with a rather high degree of species richness – evenness is a key factor in preserving the functional stability of a bacterial community under stress. It was shown that increased biodiversity resulted in an increased resistance to external forces, such invasive species.

While gradual progress is made understanding soil microbial community, little is known about the soilless microbial community. We focused in our research on the composition of the soilless community and the soilless community structure and its correlation to physico-chemical factors. However, correlation between the relative abundance of bacterial communities, physico-chemical factors and functionality does not always imply a causal relationship. The investigation of a causal relationship between bacterial abundance and function may require in depth investigation of the strength, consistency, and specificity of the correlation (Francis 2010). Molecular studies, based on 16S rRNA gene, indicate that up to 1 million different bacterial and archaeal species are present in 10 g of soil, in the context of approximately 1 billion microbial cells. This richness is not surprising, but their ecological role in soil and even more in soilless culture systems are poorly understood. In addition, our understanding of functional redundancy and of the links between microbial richness, community composition and soil-ecosystem function is far from complete, despite the promises of 16S rRNA gene-based techniques. For a more complete overview on soilless communities, advances in sequencing technology make it possible to consider tackling these issues using metagenomics and metatranscriptomics, which are defined as the characterization of all genes and transcripts, respectively, in a soil sample or a sample of an organic or mineral growing medium.

RQ2: What are the differences in microbial community composition in a mineral growing medium with plants showing the hairy roots syndrome and plants not showing the hairy roots syndrome?

Microbial community structure of a mineral growing medium was not affected as a result of an infection of egg plants by A. rhizogenes and resulting in the hairy roots syndrome compared to plants that were infected but not showing the hairy roots syndrome.

Several studies report temporal and spatial shifts in microbial communities (Rosberg 2014). Infection of cucumbers by *Pythium ultimum* resulted in different bacterial and fungal populations compared to non-infected ones when grown in compost enriched peat. Actinobacteria and α-Proteobacteria were the dominant classes in the presence of *P. ultimum*, while the control was dominated by γ-/δ-Proteobacteria. The fungal community was more greatly affected by the pathogen than the bacterial community. In our study, α-, β- and γ-Proteobacteria, such as *Hyphomicrobiaceae*, *Xanthobacteraceae*,

Phyllobacteriaceae, and *Chromatiaceae*. Actinobacteria such as *Gaiellaceae* and *Conexibacteraceae* were positively correlated with GB showing a lower pathogen infection, which is in agreement with the research of Hagn et al. (2008).

The soilless microbial community in combination with the organic growing medium was distinctly different in microbial community composition and structure, i.e. it was more diverse and even (Postma 2009). Garbeva et al. (2004) hypothesized that in a stable system, each microhabitat is occupied by organisms capable of colonizing niches. A diverse and stable ecosystem at the microhabitat level will resist environmental stresses and potentially, pathogen invasion. Mendes et al. (2011) suggested that the relative abundance of several bacterial taxa may be an indicator of disease suppression, which is in agreement with our results. In several studies the rhizosphere microbiota of diseased and non-diseased plants in soil and compost were analyzed. Zhu et al. (2013) suggests that rhizosphere bacterial community plays an important role in the changes of soybean rhizosphere biological conditions during the infection process by *Heterodera glycines*. Zhang et al. (2011) showed that bacterial communities in rhizosphere soil of healthy cotton plants at flowering and bolling stage had the highest richness, whereas the highest evenness was found in the rhizosphere of cotton at boll opening due to infection by *Verticillium dahliae* Kleb. Gardener and Weller (2001) assumed that the presence of P. ultimum induces distinct shifts in microbial communities favoring to groups known to comprise potential biocontrol agents.

RQ3: What are the differences in microbial community composition between the rhizosphere and the bulk zone?

We observed, based on MFA results, that bacterial abundances in the rhizosphere were significantly different regardless of fertilizer supplementation, especially during the early development of the plant. In the bulk zone, on the contrary, this effect was not observed. In addition, MFA showed, that the microbial community in the rhizosphere is getting more specific over time, indicated by the decreased variations in the bacterial relative abundances in the rhizosphere over time.

The effect of plant age and development stage on rhizosphere microbial communities is very well known. (Houlden et al. 2008; Micallef et al. 2009). Root exudation changes with plant age (Rovira, 1959) and consequently most likely changes in microbial community structures are caused by changes in root exudation patterns (Marschner et al. 2002; Micallef et al. 2009). Zhang et al. (2011) observed that bacterial diversity initially increased and finally decreased during plant maturation, while (Rosberg 2014) showed an increase in the bacterial species, richness and diversity with increasing plant age. We provided evidence that 1-month-old plants have a more important role on rhizosphere microbial community structure than fertilizer used in organic growing medium blended with recovered nutrients [Chapter 7]. Plant microbiome composition is affected by various host-driven factors, including the plant and fertilization (Sessitsch and Mitter 2015). Zhu et al. (2015) demonstrated that there was a minimal influence of nitrogen on rhizosphere effects, when plants grown in a nutrient-poor soil (20 or 120 µg NH_4NO_3-N g dry soil^{-1}) for 80 days. We used in our experiment [Chapter 7] higher nitrogen concentration, i.e. 550 µg N g^{-1} dry growing medium, but these recovered nutrients are not directly available for the plant and first need to undergo mineralization first in the case of the organic fertilizer. struvite can also be considered as a slow release fertilizer (Rahman et al. 2011; Rahman et al. 2014; Talboys et al. 2015). The presence of plant roots had the strongest impact (up to 80%) on rates of net N mineralization and activities of three soil enzymes indicative of nitrogen release from organic matter

(Zhu et al. 2015) in comparison with a soil without plants. This is in agreement with our results, where the presence of lupine or tomato showed a strong impact on the nitrogen and pH dynamics in the root zone. Plants secrete blends of compounds and specific phytochemicals in the root exudates that are differentially produced at distinct stages of development to help orchestrate rhizosphere microbiome assemblage, presumably for specific functions (Chaparro et al. 2014), such as ammonia oxidation. When plants and especially young plants start expanding in the growing medium, they immediately encounter the microbial community associated with the growing medium and also in a microbial rhizosphere community closely interacting with the plants (Gschwendtner et al. 2016). Our analysis showed that plant and time point significantly contributed to the differences in the relative abundances of the bacterial genera (Figure 7.6). This indicates that the bacterial abundances were significantly different, but regardless of fertilizer supplementation, especially during the early development of the plant. Many studies have shown that rhizospheric fungal and bacterial communities of a wide range of plants (i.e., *Arabidopsis*, *Medicago*, maize, pea, wheat and sugar beet) change according to a plant developmental gradient (Baudoin et al. 2002; Houlden et al. 2008; Micallef et al. 2009; Mougel et al. 2006). Chaparro et al. (2014) validated that the microbial community at the seedling stage of *Arabidopsis* was distinct from the other developmental time points. This is also in agreement with our results [Chapter 6] based on PLFA analysis. We provided evidence that plants have a more important role on rhizosphere microbial community structure based on PLFA analysis than fertilizer used in organic growing medium blended with recovered nutrients, especially at the start of the experiment.

RQ4: Are plants rather than fertilizers drivers of the microbial community composition in organic growing media blended with recovered nutrients?

MFA revealed that the impact of plant, time and sterilization on the microbial community in the bulk zone was greater than that of fertilizer. In addition, our results showed that the plant effect is determinant on the differences in the relative abundances of the communities in the rhizosphere, confirming the dissimilarity of the relative abundances between growing medium harboring different plants.

It is acknowledged that plants have an impact on soil microbial communities through carbon flow and competition for nutrients (Lynch 1994) and relatively few molecular 16S rRNA gene based studies targeted specifically at rhizosphere soils (Duineveld et al. 2001; Duineveld et al. 1998; Marilley and Aragno 1999). These studies have shown that microbial diversity in the rhizosphere is great with all of the major phylogenetic bacterial lines present within only a few millimeters of the root surface (Macrae et al. 2000). These studies also show that there are distinct differences in bacterial community structure between the bulk and the 'rhizosphere' soil. This suggests that the plant is changing microbial community structure in the vicinity of the root. However, the vicinity of the root is often interpreted as the soil adhering to the root. There are major questions in sampling procedures and this limits the identification of true rhizosphere effects from bulk zone effects. In addition, this limits our possibilities to determine the extent to which the plant selects for and regulates its own rhizosphere community. Our results indicate that plants influence the microbial community composition at a distance of more than 10 mm.

Moreover, soil and soilless culture systems differ substantially in the root volume available for the plants and this might affect rhizospheric effects. Root volume restriction in soilless culture systems

compared to non-restricted systems affect shoot growth, because of several factors. These factors are an inhibition of nutrient and water acquisition, limited oxygen supply, imbalances in growth substances between the shoots and roots, reduction in photosynthesis and changes in carbohydrate mobilization (Nishizawa and Saito 1998). Root volume restriction does not affect root to shoot ratio in peach, soybean or cucumber, indicating that shoot growth is inhibited due to restricted root growth ((Nishizawa and Saito 1998). Root restriction in tomato cultivating systems, on the contrary, results in a decreased root to shoot ratio compared to unrestricted systems, indicating a relative higher allocation of assimilates to the shoots in root restricted compared to unrestricted tomato plants. Soilless culture systems with root restriction produced approximately 0.05g g dry root weight cm^{-3} compared to 0.0012 g root dry weight cm^{-3} in the unrestricted systems (Nishizawa and Saito 1998). Moreover, root restriction had no effect on net assimilation rates (Ruff et al. 1987). The combination of a higher root dry weight per volume of growing medium and comparable net assimilation rates in root restricted systems indicate a higher impact of plants in soilless culture systems with restricted root volumes compared to unrestricted systems, i.e. soil systems.

RQ5: Do soilless culture systems with organic fertilizer and organic growing media have a higher species richness, diversity, and evenness compared to other contrasting tomato cultivation systems (GBFISH, SOILANIMAL and SOILPLANT)?

The community structure of the organic soil and the soilless culture system were distinctly and consistently different and this could be attributed to differences in chemical characteristics of the four tomato cultivating systems. Differences in diversity and evenness between the soil and soilless culture systems decreased over time mainly as a result of major community changes, i.e. increased diversity and evenness in the soilless culture system.

The diversity, structure and composition of natural communities and most likely also soilless culture communities are explained through 'niche-based' mechanisms (Chase and Leibold 2003), meaning that microbial communities are shaped by deterministic factors, such as the pH of the growing medium. In contrast, neutral theories assume all species to be ecologically equivalent (Chave 2004). Under these assumptions, species may enter or disappear from a community as a result of natural fluctuations of their abundance over time, without underlying influences of environmental conditions (Hubbell 2001). Recent research suggests that soil microbial communities are shaped by both deterministic and stochastic processes, but soil microbial communities still responded in a predictable manner to a major abiotic niche axis, soil pH, C/N ratio and the phosphorus content (Dumbrell et al. 2010). Our MFA analysis (Chapter 6) showed that the soilless culture community of GBOF was positively correlated to the abiotic variables, notably ammonium concentration, pH(H_2O) and the chloride concentration, while community of GBFISH was positively correlated to the electrical conductivity, nitrate and magnesium. This pattern was not that clear in combination with SOILANIMAL and SOILPLANT. The abiotic variables, notably nitrate and soil pH were the most significant factors determining the environmental niche of SOILANIMAL bacterial community, while phosphorus, sodium, magnesium, calcium, potassium, chloride, pH and ammonium were the most significant factors of the SOILPLANT microbial community.

The response to environmental factors of soil and soilless microbial community highlights the susceptibility of soil microbes to environmental change, but also shows the possibility to manage the

soil and most likely also the soilless communities by predefining the environment, i.e. selecting the major abiotic factors and consequently shaping the microbial community in a predictable way.

RQ6: What are the most significant physico-chemical characteristics of the soil and soilless culture systems, i.e. mineral and organic growing medium?

We identified water content, potassium content, pH(H_2O) and conductivity as the main physicochemical characteristics driving microbial communities in the different growing media. Ammonium seems to be the key N form influencing many processes, and we found that calcium, magnesium, sulphate, nitrate-N, sodium and conductivity were positively correlated to GB.

There is a clear indication that the physico-chemical environment is an important factor not only shaping the microbial community and but also influencing certain functions, such as the inhibition of the crazy roots causing pathogen by bio control agents (Bosmans et al. 2016a). Ammonium seems to be the key N form influencing many processes. In Chapter 2 we found that nitrate-N was higher in GB than in RW, while ammonium-N was significantly higher in RW in comparison with GB. Moreover, we found that ammonium-N was positively correlated with *Rhizobiaceae* abundance [Chapter 2]. The low ammonia concentration and the low pH(H_2O) in the GB medium may explain the absence of hairy roots and potentially shaped the microbial community composition. Indeed, ammonium can be produced under environmental stress (Bittsánszky et al. 2015) like pathogen attacks. In addition, plants infected by *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* can express the ammonium-producing enzyme ornithinecyclodeaminase (Sharma et al. 2013) and *Pseudomonas syringae* pv. tabaci produces tabtoxinine-β-lactam (a potent inhibitor of Glutamine synthase) in infected tissues, leading to the accumulation of phytotoxic concentrations of ammonium (Ito et al. 2014). Ammonia oxidation is the rate-limiting step resulting in an accumulation of ammonium in combination with organic fertilizers [Chapter 5-6] and with struvite [Chapter 7].

As indicated by Bosmans et al. (2016a), the nutrient composition of the agar affects *in vitro* screening of biocontrol activity of antagonistic microorganisms. A non-metric multidimensional scaling (nMDS) plot showed that Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} , Zn^{2+} and Al^{3+} separated the agars in two groups, with one group showing antagonistic activities and another not showing antagonistic activity. For the particular antagonistic interactions investigated, mentioned study suggests an important role of Ca^{2+} , which is in agreement with our results, to produce and/or secrete potential toxins/antibiotics against rhizogenic *Agrobacterium*. Whereas the importance of Ca^{2+} as a cell regulator is well established in eukaryotes (Bode et al. 2002), little is known about the precise role of Ca^{2+} in prokaryotes. Nevertheless, recent research suggests the possibility that, as in eukaryotes, Ca^{2+} plays a role in signal transduction in bacteria modulating specific functions or generating a specific response (Dominguez, 2004). The exact function of Ca^{2+} in the antagonistic interaction investigated here remains, however, to be unraveled.

3. Activity of soilless culture microbial communities

H2: In contrast to the individual medium constituents, a blend will create a more optimal physico-chemical microbial environment, resulting in higher ammonia and nitrite oxidation rates.

RQ7: Do we see an inhibition, a status quo or a stimulation of the respiration, ureolytic, ammonia and nitrite oxidation rate when individual GMC are blended with each other?

We found that the respiration, ammonia and nitrite oxidation rate was stimulated when GMC were blended with each other.

Carbon respiration rate showed significant differences between the growing media constituents tested [Chapter 3]. When the constituents were mixed together, the carbon dioxide respiration rate was increased, that might be explained by changes in the physico-chemical properties of the growing medium [Chapter 3] (Fortin et al. 1996). Carbon dioxide results from microbial, root, and faunal respiration and from non-biological chemical oxidation (Bunt 1954). In our case, microbial respiration and activity are important for organic matter mineralization. Moreover, microbial respiration can be used as a measure of community functionality (Bell et al. 2005). In general, soil microbial functional diversity and metabolic quotient (qCO_2 = soil basal respiration/soil microbial biomass) correlate inversely suggesting that an older more mature microbial community has a lower specific respiration rate. Overall respiration, however, can be higher.

Transformation rates of different growing media constituents were determined in [Chapter 3]. We found evidence for ureolytic activity in sod and Irish peats [Chapter 3]. The enrichment of the first acidophilic, autotrophic, ammonia-oxidizer, *Nitrosotalea devanaterra*, provides an explanation for nitrification in acidic soils (Lehtovirta-Morley et al. 2011). Although NOB prefer a neutral pH-value as is the case for coconut fiber, Hankinson and Schmidt (1988) succeeded in isolating a strain of *Nitrobacter* growing at pH 5.5. We found that the potential rates for ammonia oxidation and nitrite oxidation were higher for the peat-based constituents; however coconut fiber showed higher nitrite oxidation rates [Chapter 3]. The results indicate that we have different nitrifying communities in the different growing media constituents. Consequently, blending these constituents with each other indicates that we can contain a potential mix of a heterotrophic and autotrophic nitrifying community in the blend. We identified that *Nitrospira* and the *Nitrosomonadaceae* were closely related to GB [Chapter 2].

Hence, we can blend functional microbial communities associated with growing media constituents in a growing medium to get a nitrifying community in an organic growing medium. This strategy can be used as a next step towards a more sustainable horticulture in combination with soilless culture systems, where the delivery and production of organic-derived nutrients can be predicted and controlled in a more reliable way.

RQ8: What are the critical parameters for a successful nutrient management in combination with organic fertilizers?

The proper selection of a growing medium with well known chemical, physical and biological (activity) characteristics is a prerequisite. pH(H_2O), total ammonium concentration, free ammonia and dissolved oxygen are most crucial factors concerning quality control of organic growing media in combination with organic fertilizers.

Next to the efforts to identify the soilless microbial communities, the identification of critical process control parameters is also of major importance for the design of more reliable and manageable growing media in combination with organic fertilizers. To achieve a predictable and controllable use of an organic fertilizer, the total composition, i.e. N, P, K content of fertilizer needs to be known, the release of individual nutrients needs to be determined, and a standardized growing medium with known chemical, physical and biological characteristics, i.e. ammonia and nitrite oxidation rate, must

be designed. Last but not least, the mineralization curve of the organic fertilizer needs to be matched to the demand of the plant.

The release of nutrients from the organic fertilizer blended into the growing medium should be tested as much as possible under practice like conditions. We propose to set up an aerobic incubation experiment, where the organic fertilizer should be blended into a predefined growing medium (in conformity with practice) in plastic containers (9 cm, with 12 holes at the bottom, with an angle of 8°, 220 mL growing medium per plastic container) in triplicate. The organic fertilizer should be blended into the growing medium and added at a rate of 240 and 400 mg N L^{-1} (in conformity with practice) growing medium. Before filling the plastic containers, demineralized water should be added to obtain a gravimetric moisture content of 70.9 % (w/w) equivalent to a pressure potential of -10 kPa matric potential. A blank treatment should be added to determine N release from the growing medium itself during the incubation period. After thorough mixing, the plastic containers should be filled and placed in a growth chamber and covered with a gas-permeable film to minimize water loss. Whenever needed water loss should be corrected. After dag 0, 1, 5, 13 and 42 days of incubation at 21°C and 99% relative humidity, plastic containers should be destructively sampled and analyzed for mineral N content. The predefined growing medium is a mixture of black peat 20 vol.-%, coco coir 20 vol.-%, white peat 50 vol.-% and green waste compost 10 vol.-% and 3 kg lime m^{-3}. This blend has an approximate fresh bulk density of 304 kg m^{-3} or an dry bulk density of 156 kg m^{-3} and a total pore space of 0.91 m^3 m^{-3}. An overview of the different growing media constituents and its functionality connected with the mineralization of organic fertilizers can be found below in Table 8.1.

Table 8.1: Overview of the different growing media constituents and its functionality connected with the mineralization of organic fertilizers. Numbers indicate mean ± standard deviation. n=3. GMC = growing medium constituent.

GMC	Carbon respiration rate (mg CO_2-C kg^{-1} GMC d^{-1})	Potential ammonia oxidation rate (mg N kg^{-1} GMC d^{-1})	Potential nitrite oxidation rate (mg N kg^{-1} GMC d^{-1})	C/N ratio	Reference
Mineral growing media	−2.0 ± 10	ND	ND	3	(Grunert et al. 2016b)
Compost (green waste)	139 ± 20	87 ± 10	84 ± 1	16	(Grunert et al. 2016b)
Coconut fiber	83 ± 4	1.0 ± 1.0	19 ± 3	100	(Grunert et al. 2016b)
Sod peat	25 ± 2	46 ± 35	8 ± 6	60	(Grunert et al. 2016b)
Irish peat	16 ± 3	50 ± 30	18 ± 2	50	(Grunert et al. 2016b)
White peat	33 ±22	ND	59	>35	(Grunert et al. 2016b; Malmer and Holm 1984)
Composted bark	76 ± 60	ND	138	30-40	(Grunert et al. 2016b; Verdonck et al. 1983)
Black peat	13 ± 1	ND	74	20-35	(Grunert et al. 2016b; Malmer and Holm 1984)
Coco coir	2 ± 8	ND	7	130	(Abad et al. 2002; Grunert et al. 2016b)

The use of organic fertilizers also implies the need for oxygen. It can be calculated that about 4.5 g O_2 is needed per g of organic nitrogen to oxidize the organic nitrogen into nitrate. Therefore, more in depth analyses of the hydraulic conductivity and air permeability of the growing medium are needed. The hydraulic conductivity and the air permeability are the primary properties governing movement of chemicals in the aqueous and gaseous phases in growing media used in soilless culture systems. Therefore, knowledge of these properties is necessary when investigating, for instance, the mineralization and nitrification of organic fertilizers in growing media.

The oxygen saturation concentration (C_S) at 25°C is 8.3 mg O_2 L^{-1}, and increased temperatures in a glasshouse can lead to decreased oxygen concentrations and consequently impacting the nitrification. This decreased oxygen concentration leads to a smaller driving force (CS - C) and hence to a lower oxygen transfer rate (OTR): OTR = K_{LA} x (C_S-C) and consequently an accumulation of ammonium [Chapter 5]. The diffusion rate of oxygen increases with increasing temperatures, while the liquid viscosity and surface tension decreases, hereby increasing the oxygen transfer coefficient (K_{LA}). It is recommended to have DO values between 4-6 mg O_2 L^{-1} to avoid anoxic conditions.

Inhibition of *Nitrosomonas* appears at FA concentrations of 10-150 mg N L^{-1}, while *Nitrobacter* sp., responsible for the second step in nitrification, are inhibited at FA levels of 0.1-1.0 mg N L^{-1} (Anthonisen et al. 1976). Consequently, DO values below 2 mg O_2 L^{-1} should be avoided in the nutrient solution. The free ammonia (FA) concentration is depending on the pH, the temperature (T) and the total ammonium concentration and can be calculated according to Anthonisen et al. (1976).

$$\text{Free ammonia (mg N/l)} = \frac{Total\ ammonium\ nitrogen\left(mg\frac{N}{L}\right) x\ 10^{pH}}{e^{6344/(273+T)}+10^{pH}}$$

RQ9: Can we use commercial available organic fertilizers and to what extent do we need to adapt the N fertilization strategy and can we estimate the risk of ammonium toxicity?

Adapted fertigation strategies in combination with organic fertilizers are needed not only with respect to plant growth, but also due to the fact that the mineralization of the organic nitrogen is a biological driven process. Our results indicate that the use of organic fertilizers in combination with soilless culture systems and organic growing media require loads close to the ammonia oxidation rate of the blend (83 mg N kg $^{-1}$ d^{-1}) with a maximum concentration of 315 mg N L^{-1} in order to allow a community shift towards a more adapted nitrifying community and avoid ammonium toxicity.

However, some plants like sweet basil (*Ocimum basilicum*) require high amounts of nitrogen (600-800 mg N L^{-1} of growing medium) that needs to be blended into the growing medium to meet the nitrogen demand of the plant. This may result in a high electrical conductivity due to mineralization of the organic fertilizer and jeopardizing the germination and plant growth. This problem can be solved by mixing a maximum amount of 240 mg N L^{-1} of growing medium and supplying the plant with an liquid organic fertilizer on a regular basis thereby respecting the step-wise increase of the nitrogen supply rate (Chapter 5).

The removal of ammonium ions from effluents has become almost a necessity. Experimental results of Haralambous et al. (1992) showed that the use of zeolite is an attractive and promising method for ammonium removal. Consequently, clinoptilitic zeolites are promising additives in organic growing

media in combination with organic fertilizer to remove excess ammonium and thereby avoiding ammonium toxicity.

4. Novel soilless culture strategies to close the yield gap in soilless culture systems in combination with organic fertilizers

H3: In tomato soilless cultivation systems, a gradual increase of the organic nitrogen supply rate will result in comparable yields compared to inorganic fertilizers.

RQ10: What is the effect of a gradual increase of the organic nitrogen supply rate on the yield and the quality of the tomatoes compared to constant inorganic nitrogen supply rate?

We demonstrated in a proof of concept experiment that a step-wise increase of the organic N-supply rate resulted in comparable yields as a conventional system with a limited accumulation of free ammonia and ammonium levels potentially toxic for the plant.

Numerous individual studies have compared yield differences in the soil between organic and conventional systems. According to these studies, yield averages are 8 to 25% lower in soil organic systems compared to conventional soil systems (Reganold and Wachter 2016). In soilless culture systems there is only limited information available. Heeb et al. (2005a) compared organic fertilizers, based on chicken manure or fresh grass clover mulch, to mineral fertilizer nutrient solutions with ammonium or nitrate as the dominant nitrogen source in sand as a growing medium. In both years yields from the mineral fertilized tomato plants were higher (12-21%) than from the organic fertilized ones. It was concluded that organic or mineral fertilizers are not the major factors affecting yield and product quality. Heeb et al. (2005a) supplied the tomato plants with the same nitrogen supply rate throughout the whole experimental setup of 11 weeks, which is comparable to our plant test. Tomato plants were supplied with 250, 500, 750 and 1000 mg N $plant^{-1}$ d^{-1} and in our plant test plants nitrogen supply rate was increased from 73 mg N $plant^{-1}$ d^{-1} , 142 mg N $plant^{-1}$ d^{-1}, 218 mg N $plant^{-1}$ d^{-1} and finally 331 mg N $plant^{-1}$ d^{-1}. This resulted in equal tomato yield between the control treatment and the organic fertilizer treatment. These results indicate that next a balanced nutrient supply, a step wise increase of the nitrogen supply rate is an important strategy to obtain high yield and quality.
A combination of organic and mineral fertilizers should be considered in order to achieve a resource saving and balanced nutrient supply and a high quality tomato yield. For example, integrated farming systems that blend mostly organic with some conventional practices have been shown to be more sustainable than conventional farming systems and are likely to play a central role.

H4: Novel recovered fertilizers can replace conventional fertilizers resulting in a comparable plant performance (yield and quality).

RQ11: What is the effect of these recovered nutrients on plant performance (yield and quality)?

The use of advanced nutrient recycling technologies and green fertilizers is pivotal in the transition towards a more sustainable and resource-efficient food production system. This study demonstrates that microalgae biomass, organic fertilizers and struvite can be used as fertilizer for tomato cultivation.

The microalgae fertilizers improve the quality of the fruits produced through an increase in the sugar and carotenoid content of fruits. Further research is required to determine optimal fertilizer mixtures that produce high quality fruits with satisfactory yields compared to conventional systems with inorganic fertilizers.

Microalgae-based fertilizers offer advantages within a larger sustainability framework [chapter 4]. In contrast to nutrient-rich waste streams such as manure, microalgae biomass can function as a stable, predictable, transportable and concentrated fertilizer product (Coppens 2016). The additional plant growth-promoting characteristics of the microalgae biomass demonstrated that nutrients recovered through microalgae can give an added value compared to the direct application of waste streams on cropland (Mulbry et al. 2007). The production of microalgae biomass from waste streams could, therefore, transform waste nutrients into sustainable high-value fertilizers with commercial relevance in glasshouse horticulture systems. Several commercially available organic fertilizers [chapter 4-7] were tested for the cultivation of tomato plants and it was confirmed that they can be used as a fertilizer. However it needs to be taken into account that adapted fertigation strategies are needed [Chapter 5] and a more balanced nutrient composition is needed with respect to the use of struvite [Chapter 7].

Globally, tomato is one of the most produced vegetables, ranking second after potato (Kumari et al. 2011). This illustrates the economic and nutritional importance of this crop. The increased sugar and carotenoid concentrations obtained with the algal fertilizers [Chapter 4] indicate the potential of microalgae-based fertilizers to increase the quality and economic value of tomato fruits. Carotenoids play an important role in many plants during photosynthesis, the protection against photo-oxidative stress and attraction of insects. The amendment with microalgae biomass can, therefore, also have beneficial effects for other high-value plants. Comparable to tomatoes, microalgae fertilizers might improve the value of peppers (*Capsicum annuum*), while they might also be implemented in flower cultivation, as carotenoids induce the typical yellow and orange color in for instance roses (Lachman et al. 2001). The use of recovered nutrients, such as organic fertilizers demands adapted fertigation strategies. Chapter 5 shows that a step wise increase of the nitrogen-load is a promising approach to narrow the yield gap between inorganically and organically grown vegetables and represents the basis for prolonged field trials with tomatoes. These results support our hypothesis that we need to accustom the plant to high concentrations of organic derived nitrogen and have a functional microbial community associated with the growing medium. These results were also confirmed by a tomato cultivating experiment during a whole season (February 2015 till November 2015) in which it was shown that organic fertilizer could be used for the cultivation of tomato plants. The actual bottleneck of the organic fertilization for glasshouse vegetable crops is their unbalanced nutrient composition resulting in the build-up up of salinity and an unbalanced soil nutrient solution - too much phosphorus, sodium, chloride, sulphur and not enough potassium and nitrogen.

Nevertheless, the difference in fruit yield in combination with recovered nutrients compared to the conventional horticulture fertilizers indicates that a more optimal fertilizer mixture and or a fertigation strategy is required to combine high quality fruits with satisfactory yields [Chapter 4 and 6]. The addition of microalgae biomass as an additive might improve the market value of the products [Chapter 4]. Although further research is required to assess the optimal amount of microalgae that needs to be blended in the growing medium, previous findings related to the application of phototrophic organisms as bio fertilizers suggest that the microalgae biomass might have beneficial

effects on crop output (Kumari et al. 2011; Tripathi et al. 2008). Microalgae-based fertilizers can be applied in an Integrated Plant Nutrition System (IPNS). This integrated cultivation concept combines and optimizes the use of inorganic, organic and bio fertilizers to sustain desired crop productivity with a minimal impact on the environment (Chen 2006).

Novel recovered fertilizers are promising, however consumer acceptance (B2B and B2C) is of major importance. Lienert et al. (2003) studied the acceptance of a urine-based fertilizer product using a mail survey of 467 Swiss farmers. Mentioned study distinguished among four production types: organic or conventional farming, and with or without vegetable production. Almost 57% of the surveyed people explicitly stated that they thought it was a good or very good idea, and 42% willing to purchase such a product. Especially conventional farmers and vegetable farmers were willing to accept urine-based fertilizers, hereby preferring a grainy, odorless ammonium nitrate fertilizer. Essential is a hazard-free product: 30% of all farmers had concerns regarding micro pollutants. Indeed this is a major point of attention with respect to the acceptance of these novel recovered nutrients.

RQ12: Is it economic feasible to use microalgae fertilizers in comparison with conventional inorganic and organic growing media in soilless culture systems?

Microalgae fertilizers are not feasible when applied as the main nitrogen fertilizer source

The economic practicality of recovered nutrients, such as microalgae-based and organic fertilizers for horticultural applications was assessed through different fertilizer scenarios [Chapter 4]. The use of microalgae was thereby combined with inorganic and organic commercially available fertilizers for glasshouse tomato cultivation. All detailed economic information (Supplementary table 8.1, Supplementary table 8.2 and Supplementary table 8.3) is based on the joint paper of Coppens et al. (2015). Commercial inorganic NPK fertilizers cost € 7.9 kg^{-1} N, while organic fertilizers with 4% and 8% of organic nitrogen cost € 10.5 kg^{-1} N and € 7.3 kg^{-1} N, respectively. Controlled release fertilizers cost up to € 33 euro kg^{-1} N. This indicates that microalgae biomass, as such, is not economically competitive with commercial horticulture fertilizers (€ 893 euro kg^{-1} N) -even the controlled release fertilizers- when applied as the main nitrogen fertilizer source.

A cost assessment of glasshouse tomato cultivation shows that tomato production using inorganic and organic fertilizers comes at a total production cost of € 0.73 kg^{-1} fruit and € 1.10 kg^{-1} fruit, respectively (Figure 8.1). Labor contributes 38% and is the predominant cost factor, while fertilizers only account for 3% to the total production cost. The low economic impact of the use of fertilizers therefore allows within a certain range for more expensive fertilizers to be incorporated if an economic added value can be delivered through for instance superior fruit quality and compensate for the increased cost of the fertilizer.

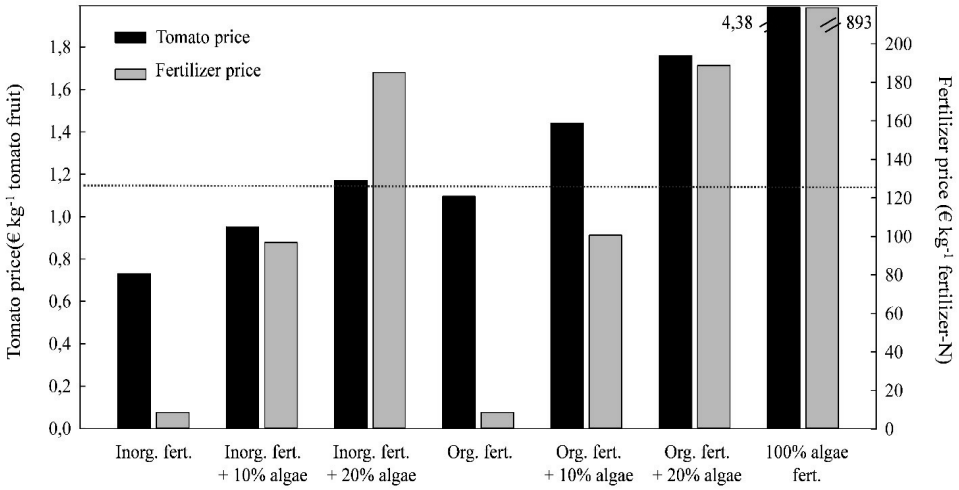

Figure 8.1: Cost of different fertilizer mixtures of microalgae and inorganic and organic fertilizers and the respective production cost of the tomato fruits. The dotted line indicates the average auction price of organically grown tomatoes in 2013-2014 (€ 1.16 kg^{-1}; PCG, personal communication)

On average, the economic value of auctioned organically grown tomatoes is 33% higher than those obtained through conventional inorganic cultivation (Auction Hoogstraten SCRL (2015); Provincial Center for Crop Cultivation, Kruishoutem, personal communication). In the case where a comparable price would be obtained for tomatoes grown on inorganic and microalgae- based fertilizers, microalgae could be blended in for up to 17% of the nitrogen demand of the plants while remaining economically competitive (Figure 8.1). The amendment of 10% of the nitrogen demand of the plant as a microalgae fertilizer to a conventional inorganic fertilizer system could potentially combine the improved plant growth of nitrate-based inorganic fertilizers with the beneficial effects on fruit quality of microalgae. This system would result in a 34% increase of the production cost. Although is still to be tested in practice, the higher production cost might be balanced by the added market value due to improved taste and pigmentation which could allow to charge a premium price similar to organic produce. It needs to be considered, that this economic evaluation does not take into account the additional savings, which can be obtained within the glasshouse cultivation system through on-site microalgae production or even the clustering of activities with mutual interest (Tomatomasters and Aqua4C, Kruishoutem, Belgium). The production of microalgae allows for the recovery of excess nutrients present in the glasshouse effluents, thereby reducing or even eliminating discharge to the environment and enabling the reclamation of water for further use. This would improve production yields and hence reduce the total production costs.

RQ13: How is the use of recovered nutrients, such as ammonium struvite, regulated at national and European level?

The use of fertilizers, soil improvers and growing media in soilless culture systems is governed by national (KB 2013) and EU legislation. Until now struvite is not included, while organic fertilizers are included in the list of approved fertilizers

The use of fertilizers, soil improvers and growing media in soilless culture systems is governed by national (KB 2013) and EU legislation. According to the national legislation, a fertilizer, soil improver, a growing medium, sewage sludge or comparable products may be traded in Belgium if it is classified as a mineral fertilizer (according to regulation 2003/2003, annex I). In Belgium, an individual exemption must be approved by the Belgian Federal Public Service Public Health, which considers the product description, composition, and application, together with the production legislation of the neighboring countries (Belgisch staatsblad 2013). The third possibility is that the fertilizer, soil improver of growing medium to certify the fertilizer according regulation (EG) Nr. 764/2008 (mutual recognition) from the European parliament and the European council from July 9th 2008). According to the national legislation (KB2013) growing media should have a dry matter content of 20%, an organic matter content of 50% of the dry matter content, a pH (H_2O) between 3,5 and 5 for acidophilic plants, a pH (H_2O) between 6,5 and 7,5 for alkaline plants and a pH (H_2O) between 4,5 and 7 for other plants. The electrical conductivity should not exceed 750 µS cm^{-1}. In March 2016, the European Commission published its Proposal for a Regulation on CE marked fertilizing products, which contains new rules for the harmonization of growing media and soil improvers.

According to the European organic Regulations (EC 834/2007, 889/2008 and 1235/2008) the application of algal, fungal and bacterial biomass products is allowed in organic horticulture, regardless of the means of production. The EU Fertilizer Regulation 2003/2003 (EC 2003) is currently under revision. According to a press release by the European Commission (17th of march 2016) about the "Circular economy: New Regulation to boost the use of organic and waste-based fertilizers" this revision is uppermost needed, because the reuse of raw materials that are now disposed as waste is one of the key principles of the Circular Economy Package adopted in December 2015. More and more manufacturers in the EU are developing innovative fertilizing products including nutrients or organic matter recycled from bio-waste. But diverging national rules and standards make it difficult for producers of organic fertilizers to sell and use them across the EU single market. The existing fertilizer regulation ensures a free movement for traditional fertilizers typically made of mined or synthetic raw materials but it does not include a clearing procedure for organic fertilizers. As a result, around half of all fertilizers produced currently stay in the country where they were produced. This is the case for virtually all fertilizers produced from organic materials, such as animal by-products or other agricultural residual products, or recycled bio-waste. Inorganic fertilizers have therefore a competitive advantage which hampers innovation and investment in the circular economy. The Commission therefore proposes to bring organic fertilizers within the scope of the Fertilizers Regulation and grant access to CE marking. The new Regulation will create a level playing field for all fertilizing products. At the same time, new common requirements for quality, safety and labelling will guarantee a high level of safety and environmental protection of all CE marked fertilizing products. All products will be labelled in a uniform way allowing European farmers to make informed choices, contributing to making food production more cost and resource effective" (Delvaux 2016).

RQ14: EU organic farming legislation in relation to soilless culture systems and growing media

EU organic farming legislation (EC No 889/2008) forbids hydroponic (soilless culture systems) production. Hydroponic products are defined as a “method of growing plants with their roots in a mineral nutrient solution only or in an inert medium, such as perlite, gravel or mineral wool to which a nutrient solution is added”. EU legislation specifically allows the use of organic growing media for organic farming. However, it currently does not contain any specific rules.

Each EU member state currently certifies a different range of products grown in organic growing media as EU organic. Growing medium constituents (GMC), such as compost, peat, composted bark, etc. are specifically allowed in EU organic farming (see Annex 1 of mentioned EU legislation) and play an important role for Europe’s agriculture. These materials are mixed into the soil to improve conditions for plants as so-called “soil improvers” (= “soil conditioners” in the current legal text) and used to provide an optimum rooting environment for plants by supplying roots with nutrients, air, water, and physical stability – this growing technique is called growing in growing media.

Moreover, the European commission seems to allows the organic production of chicory in water, growing media and soil (personal communication by An Jamart, bioforum 29 September 2016). The EGTOP Group (Expert Group for Technical Advice on Organic Production, 11 September 2016) recommends that growing in growing media is accepted for seedlings and transplants, and for plants which are sold to the consumer together with the pot/container in which they grow (e.g. herbs in pots, ornamentals), while harvested organic vegetables or fruits (e.g. strawberries) should come from plants grown in the soil, and not from soilless culture systems. However, the EGTOP Group makes an exception for the growing of vegetables in growing media in demarcated beds in the future for farms which grew such cultures before 2013 in Finland, Sweden, Norway and Denmark, on the condition that the growing media and plastic is recycled. In fact, the Group recommends that any excess or spent growing media or plastic used in organic greenhouse production and farming in general should be reused or recycled.

Currently, the organic hydroponic production is allowed within USDA NOP (The National Organic Program is a regulatory program housed within the USDA Agricultural Marketing Service) as long as the producer can demonstrate compliance with the USDA organic regulations. The PuraNatura Foundation believes that European Organic legislation should not be developed in "splendid isolation". The regulations concerning organic farming in the European and North American market are already declared being "equivalent" and current differences may be used to improve both systems collectively. Such convergence also allows producers outside the mentioned areas to enter the system more easily. 4Evergreen/Grootscholte is a grower in the Netherlands, that uses the PuraNatura growing concept and they produce USDA NOP Organic certified vegetables for organic market in the USA according USDA organic regulations. The Grootscholte Family, renowned bell-pepper growers in the Netherlands and Spain have been awarded the Dutch Tuinbouwondernemersprijs 2014 for their outstanding business development in both conventional and organic cultivation.

5. Suggestions for future research

The Next Green Revolution may rely on microbes (Graber 2014). By 2030, the UN’s Food and Agriculture organization predicts food demand will increase by 35%. The IPCC’s latest report on the subject (Pachauri et al. 2014), shows that scientists are predicting a 2% decrease in crop yields per

decade over the next century. Selective breeding doesn't seem to be offering the types of dramatic yield increases seen in the past (Graber 2014). Gardening with the microbial community in soilless culture systems or the interphase prokaryotic/eukaryotic interaction is promising and seems to be booming business for decades to come (personal communication by Prof Verstraete). Many scientists are saying that we've been looking at the wrong set of genes (Graber 2014). Instead of in plants, the crucial genes may reside in the galaxy of bacteria and fungi that live in the soil and the growing media.

Can we identify and transfer/transplant a whole endophyte microbiome from soil in soilless culture systems? Can we inoculate plants growing in an organic growing medium with a whole endophyte microbiome and make it use less water and endure higher temperatures?

Microorganisms in the soil and growing media function much like the human microbiome, which helps us break down food, access nutrients, and defend against harmful invaders. As indicated by Rodriguez and Redman (2008), more than 400 million years of evolution and some plants still can't make it on their own. Bell et al. (2005) suggested not to transfer genes but a whole endophyte microbiome, i.e. a set of evolved species and wheat can use 50% less water and can endure 70 °C. The interphase of plants and soil or growing media is 'gardened' for instance in the roots by exudates (Guttman et al. 2014). It has been shown that the plant rhizosphere contains a resistant community and that the microbial community, that becomes established on the root remains with the plant even when the plant is placed into soil with a vastly different microbiota (Turnbull et al. 2014). This complex plant-associated microbial community, also referred to as the second genome of the plant, is also crucial for plant health (Berendsen et al. 2012).

New innovative soilless culture cultivation systems are needed for the cultivation of plants making use of organic growing media, recovered nutrients, such as organic fertilizers and a microbiome with a higher level of organization and functionality, i.e. mineralization, ammonia and nitrite oxidation in close cooperation with the plant and the growing medium. Consequently, the need to understand the "microbiome," while simultaneously being able to manage them, may provide us with more sustainable solutions for the future and will be a booming business for decades to come.

Can we use organic fertilizers in vertical farming systems for the growth of lettuce in combination with organic growing media with a low air permeability? Do we need bio reactor based technologies to convert the organic derived nitrogen in plant available inorganic nitrogen.

In addition, this knowledge could be further expanded and adapted to other soilless culture techniques of fruit and vegetable production. The horticultural West European market is a mature market that shows a fast consolidation. However more and more fruits and vegetables are grown out of the soil. Lettuce and herbs are grown more and more out of soil and for this application special growing media, i.e; blocking compost, are needed. These growing media however are characterized by a very low air content and air permeability hindering the nitrification. Hence new solutions are needed to facilitate nitrification in these low air content environments. Fruits like strawberries, blueberries, and cranberries are becoming more and more popular and with soilless culture systems yields of up to 60-70t ha^{-1} are possible with an increased quality in comparison with the soil with up to 25 t ha^{-1}. The use of microalgae could be used for the production of new "superfoods. New urban farming systems are developed and the direct contact with the grower becomes more and more important and there is an increased need for professionality and innovation and technology.

Supplementary information

Economic evaluation of algae-based fertilizers

Different fertilizer scenarios were evaluated for their economic feasibility. For these scenarios the tomato fruit yield and fertilizer price differed, whereas other fixed and variable costs were assumed to be the same (Supplementary table 8.3). General fixed and variable costs of glasshouse tomato cultivation were obtained from a government survey glasshouse tomato farmers in Flanders, Belgium (Jourquin et al. 2013). Additional production data was obtained from DLV Plant (personal communication).

Supplementary table 8.1: Overview the of variable and fixed costs of glasshouse tomato cultivation, excluding fertilizer costs

	Value (€ m^{-2})	Assumptions	References
Variable costs			
CO_2 (consumption)	2.6	46 kg CO_2 m^{-2}	DLV Plant (personal communication)
heat	4.0	1548 MJ m^{-2}	DLV Plant (personal communication)
Electricity	0.6	80 W m^{-2}	DLV Plant (personal communication)
tomato plants	3.2	3.3 plants m^{-2}	(Jourquin et al. 2013)
crop protection	0.5		(Jourquin et al. 2013)
growing medium	0.8	The same price for inorganic and organic growing medium is assumed	(Jourquin et al. 2013) Peltracom (personal communication)
support and binding materials	0.5		(Jourquin et al. 2013)
other variable costs (incl. waste management)	1.0		(Jourquin et al. 2013)
marketing	0.9	2% of turnover	DLV Plant (personal communication)
transportation costs	0.2	0.4% of turnover	DLV Plant (personal communication)
labor	14.45	875 hr./1000 m² at €16.5/hr.	DLV Plant (personal communication)
Equipment & maintenance costs	0.5		(Jourquin et al. 2013)
Fixed costs			
Costs of buildings	4.0		(Jourquin et al. 2013)
Other fixed costs (insurances, taxes,..)	0.9		(Jourquin et al. 2013)
depreciations	3.2		(Jourquin et al. 2013)
Total costs	**37.2**	excluding fertilizers	

A plant nutrient requirement of 133g N m^{-2}, 34 g P_2O_5 m^{-2} and 233 g K_2O m^{-2} is assumed according to commercial glasshouse tomato production practices (Haifa 2012). For inorganic fertilizer scenarios the commercial NPK fertilizer is amended with K_2SO_4 to provide the additional potassium demand, while the organic fertilizer scenarios are amended with kali vinasse (38% K_2O; Rendapart, Belgium). A tomato production yield of 52 kg m^{-2} yr^{-1} is assumed for the inorganic fertilizer treatments, according to average production yields in Flanders (Jourquin et al. 2013). A yield of 35 kg m^{-2} yr^{-1} is assumed for the different organic fertilizer treatments (Dewitte et al. 2013).

The cost for microalgae production is obtained from Coppens (2016) and is based on the economic evaluation of microalgae cultivation in an outdoor raceway pond according to (Norsker et al. (2011)). A microalgae production cost of € 23 kg^{-1}biomass or € 288 kg^{-1}N is obtained. Considering a 33% N mineralization of the microalgae biomass, a fertilizer cost of € 871 kg^{-1} fertilizer-N is obtained.

Supplementary table 8.2: Price overview of different inorganic and organic fertilizers

Fertilizer	**Price (€/kg)**	**Reference**
Inorganic NPK fertilizer (14% N, 7% P, 15% K)	1.1	Peltracom
K_2SO_4 (43% K)	0.65	Peltracom
organic slow-release fertilizer SF1 (4% N, 2.2% P, 5% K)	0.42	Peltracom
organic slow-release fertilizer SF2 (8% N, 2.2% P, 5% K)	0.58	Peltracom
Kali vinasse (32% K)	0.88	Peltracom
Microalgae (8% N, 1.3% P, 0.2% K)	23	

Supplementary table 8.3: Overview of the production costs for the different fertilizer scenarios

	Inorganic fertilizer	**Inorganic fertilizer + 10% algae**	**Inorganic fertilizer + 20% algae**	**organic fertilizer**	**organic fertilizer + 10% algae**	**organic fertilizer + 20% algae**	**100% algae fertilizer**
Yield (kg m^{-2})	52.4	52.4	52.4	35	35	3 5	35
Costs excl. fertilizer (€ m^{-2})	37.2	37.2	37.2	37.2	37.2	37.2	37.2
Fertilizers (m^{-2})	0.95 kg inorganic 0.12 kg K_2SO_4	0.50 kg algae 0.85 kg inorganic 0.13 kg K_2SO_4	1.01 kg algae 0.76 kg inorganic 0.15 kg K_2SO_4	2.46 kg SF1 0.69 kg SF2 0.18 kg K-vinasse	0.50 kg algae 3.32 kg SF1 0.13 kg K vinasse	1.01 kg algae 2.95 kg SF1 0.18 kg K-vinasse	5.03 kg algae 0.54 kg K-vinasse
Fertilizer cost (€ m^{-2})	1.12	12.60	24.06	1.13	13.10	24.5	116.1
Fertilizer cost (€ kg^{-1} fertilizer-N)	8.62	96.92	185.08	8.52	100.6	188.7	893.3
Total cost (€ m^{-2})	38.33	49.80	61.26	38.3	50.3	61.7	153.3
Total cost (€ kg^{-1} tomato)	**0.73**	**0.95**	**1.17**	**1.10**	**1.44**	**1.76**	**4.38**

ABSTRACT

The soilless glasshouse systems rely heavily on increased yields. These soilless culture systems, however, generate every year enormous volumes of waste that needs to be recycled, uses a high amount of inorganic fertilizers and they start with a 'microbiological vacuum', *i.e.* they lack a diverse microbial community. This prompted the search for novel growing media, which could be composted at the end of the growing season and could be used for the cultivation of tomatoes in glasshouse horticulture. Six research chapter were elaborated in this study, with the aim to investigate the GROWING MEDIUM–NUTRIENTS-MICROBIAL COMMUNITY-PLANT interaction. This knowledge is used to develop sustainable soilless tomato cultivating systems in combination with organic growing media, recovered nutrients, such as organic fertilizers and ammonium struvite and a functional microbial community.

In [Chapter 2], the microbial community composition associated with a mineral and an organic growing medium in a soilless culture system was investigated. In addition, we studied the effect of an infection of the plants by the hairy roots causing pathogen A*grobacterium rhizogenes* on the microbial community composition. High throughput sequencing analysis revealed a distinctive and stable microbial community in the organic growing medium. Water content, pH(H2O), nitrate-N, ammonium-N and conductivity were the main physico-chemical factors associated with the resident bacterial communities. Ammonium-N was correlated with *Rhizobiaceae* abundance, while potential competitive interactions between both *Methylophilaceae* and *Actinobacteridae* with *Rhizobiaceae* were suggested. Our results revealed that soilless growing media have unique niches for diverse bacterial communities with temporal functional stability, which may positively impact the resistance to *Agrobacterium rhizogenes* causing the "hairy roots syndrome".

In [Chapter 3], the interaction between nutrients, the microbial community and several growing media constituents was examined. We demonstrated in [Chapter 3] that growing media constituents showed differences in urea hydrolysis, ammonia and nitrite oxidation and in carbon dioxide respiration rate. Mixing of the growing media constituents increased the ammonia oxidation rate from 41 mg N kg^{-1} d^{-1} to 83 mg N kg^{-1} d^{-1} and increased the nitrite oxidation rate from 15 mg N kg^{-1} d^{-1} to 63 mg N kg^{-1} d^{-1}. The use of organic fertilizer resulted in an increase of ammonia oxidizing bacteria by factor 100 compared to inorganic fertilizers. These results support our hypothesis that the activity of the functional microbial community with respect to nitrogen turnover in an organic growing medium can be improved by selecting and mixing the appropriate growing media components with each other.

The aim of [Chapter 7] was to validate the use of recovered nutrients, such as organic fertilizers and struvite on young (<35 days old) tomato and lupine plants. The use of struvite as N fertilizer resulted in a decrease of the leaf area by 28% in comparison to the organic fertilizer. Multivariate analysis showed that plants rather than fertilizer drive rhizosphere bacterial community in organic growing medium blended with recovered nutrients. Ammonia oxidation rates were 38% lower in the rhizosphere in comparison with the bulk zone. Fertilizer type and the interaction between plant and fertilizer type impacted species richness, diversity, and evenness.

The focus of [Chapter 4] was to explore the possibilities of microalgal biomass and recovered nutrients as plant fertilizer. Microalgal bacterial flocs, marine microalgae and organic fertilizers were used as an

organic slow release fertilizer for tomato cultivation. The inorganic fertilizer treatment showed higher mean plant length in comparison with the organic treatment, MaB-flocs and *Nannochloropsis* treatment over the whole experimental period. Nevertheless, there was no significant difference in the final plant height for the organic fertilizer, MaB-flocs and *Nannochloropsis* treatment. Furthermore, the microalgae fertilizers improved significantly the fruit quality through an increase in sugar and carotenoid content, although a lower tomato yield was obtained.

[Chapter 4] showed the high potential of recovered nutrients as fertilizers, however, it was concluded that further research is needed to optimize the fertigation strategy with recovered nutrients [Chapter 5] and try to close the yield gap [Chapter 4]. We investigated how a step-wise increase of the organic nitrogen load affects the growth performance of tomato plants in combination with a mineral and an organic growing medium in an independent glasshouse trial. We demonstrated that a step-wise increase of the N-load is a promising fertigation strategy resulting in comparable yields compared to a conventional system. This step wise increase of the nitrogen load produced limited amounts of free ammonia and ammonium levels potentially toxifying the plant.

Finally, [in Chapter 6], we compared in a tomato cultivating experiment throughout a whole season (Feb till November) the chemical and microbial community characteristics of four tomato cultivation systems (soil versus soilless and organic versus inorganic fertilization) and the growth of tomato plants and yield. Phospholipid fatty acid profiling (PLFA) was used to unravel the complex microbial interaction. We demonstrated that the four different cultivating systems showed differences in microbial community composition. The soilless culture system in combination with organic fertilizer showed a yield gap of 15% in comparison to the inorganic control. The use of organic growing medium in combination with an organic fertilizer is a promising approach to move towards a more sustainable horticulture. Nevertheless, additional tomato cultivating experiments are needed to confirm these results.

SAMENVATTING

Grondloze kassystemen hangen in sterke mate af van hoge teeltopbrengsten. Deze grondloze systemen produceren ieder jaar enorme hoeveelheden afval dat moet gerecycleerd worden, ze verbruiken een grote hoeveelheid minerale meststoffen en ze starten met een 'microbieel vacuüm' bv. ze hebben geen gediversifieerde microbiële gemeenschap. Dit stimuleert het zoeken naar een nieuw groeimedium, dat kan gecomposteerd worden op het einde van het groeiseizoen en kan hergebruikt worden voor de teelt van tomaten in de glastuinbouw. Zes onderzoekhoofdstukken werden op deze studie uitgevoerd met als doel de interactie GROEIMEDIUM – VOEDINGSTOFFEN – MIRCOBIELE GEMEENSCHAP – PLANT te onderzoeken. Deze kennis wordt gebruikt om een duurzaam grondloos tomatenteeltsysteem te ontwikkelen in combinatie met organische groeimedia, gerecycleerde voedingstoffen zoals organische meststoffen en ammoniak struviet en een functionele microbiële gemeenschap.

In [Hoofdstuk 2] werd de samenstelling van de microbiële gemeenschap onderzocht gelinkt aan een mineraal en organisch groeimedium in een grondloos systeem. Bovendien onderzochten wij het effect van de plantinfectie met het 'gekkewortelsyndroom' veroorzakende *Agrobacterium rhizogenes* op de samenstelling van de microbiële gemeenschap. 'High throughput sequencing analyse' toonde een karakteristieke en stabiele microbiële gemeenschap in een organisch groeimedium. Vochtgehalte, pH(H_2O), nitraat-N, ammonium-N en geleidbaarheid waren de belangrijkste fysico-chemische factoren verbonden met de aanwezige bacteriële gemeenschappen. Ammoniakale stikstof was gecorreleerd met *Rhizobiaceae* abundance, terwijl potentiële competitieve interactie werd gesuggereerd tussen zowel *Methylophilaceae* als *Actinobacteridae* met *Rhizobiaceae*. Onze resultaten tonen aan dat de grondloze groeimedia unieke niches hebben voor verschillende bacteriële gemeenschappen met een tijdelijke functionele stabiliteit en een positieve invloed kunnen hebben op de weerstand tegen het door *Agrobacterium rhizogenes* veroorzaakte 'gekke wortelsyndroom'.

In [Hoofdstuk 3] werd de interactie onderzocht tussen voedingstoffen, de microbiële gemeenschap en verschillende groeimediumbestanddelen. We toonden in [Hoofdstuk 3] aan dat groeimediabestanddelen verschillen in ureumhydrolyse, ammoniak- en nitraatoxidatie en in CO_2-ademhalings hoeveelheid. Het mengen van groeimediabestanddelen verhoogde de ammoniakoxidatie hoeveelheid van 41 mg N kg^{-1} d^{-1} tot 83 mg N kg^{-1} d^{-1}en verhoogde de nitrietoxidatie hoeveelheid van 15 mg kg^{-1} d^{-1} tot 63 mg kg^{-1} d^{-1}. Het gebruik van organische meststoffen resulteerde in een toename van de ammoniak oxidatie bacteriën met een factor 100 in vergelijking met minerale meststoffen. Deze resultaten ondersteunen onze hypothese dat de activiteit van een functionele microbiële gemeenschap t.a.v. de stikstofomzetting in een organisch groeimedium kan verbeterd worden via de selectie en het mengen van geschikte groeimediumbestanddelen.

Het doel van [Hoofdstuk 7] was het valideren van het gebruik van gerecycleerde voedingstoffen, zoals organische meststoffen en struviet op jonge (<35 dagen) tomaten- en lupineplanten. Het gebruik van struviet als N-meststof resulteerde in een afname van de bladoppervlakte met 28% in vergelijking met organische meststoffen. Multivariabele analyse toonde aan dat planten eerder dan de meststoffen de microbiële gemeenschap sturen in de rhizosfeer in een groeimedium gemengd met gerecycleerde voedingstoffen. De ammoniak oxidatie hoeveelheden waren 38% lager in de rhizosfeer dan in de bulkzone. Bemestingstype en de interactie tussen plant en meststoftype beïnvloedde de species rijkdom, de diversiteit en de gelijkmatigheid.

De focus van [Hoofdstuk 4] was het onderzoek van de mogelijkheden van micro-algen biomassa en gerecycleerde voedingstoffen als plantmeststof. Bacteriele vlokken op basis van micro-algen (Mab vlokken), mariene micro-algen (*Nannochloropsis*) en organische meststoffen werden gebruikt als een traag werkende organische meststof voor de teelt van tomaten. De minerale bemesting gaf over de gehele proefperiode heen een gemiddeld hoger plantlengte in vergelijking met de organische bemesting, MaB vlokken en Nannochloropsis-behandeling. Er was geen statistisch betrouwbaar verschil in de eindhoogte van de planten tussen de organische bemeste, MaB-vlokken en Nannochloropsis-behandeling. Bovendien verbeterde de micro-algen bemesting statistisch betrouwbaar de vruchtkwaliteit via de verhoging van het suiker- en carotenoïde-gehalte doch anderzijds werd een lager tomatenopbrengst bekomen. [Hoofdstuk 4] toonde de grote mogelijkheden aan van gerecycleerde voedingstoffen als meststoffen alhoewel verder onderzoek nodig is om de bemestingsstrategie te optimaliseren met gerecycleerde voedingstoffen [Hoofdstuk 5] en om het opbrengstverlies uit te schakelen [Hoofdstuk 4]. We onderzochten in een plantenproef hoe een stapsgewijze toename van de organische N-belasting de groeiontwikkeling beïnvloedde van tomatenplanten in combinatie met een mineraal en organisch groeimedium. We toonden aan dat een stapsgewijze toename van de N-belasting in een beloftevolle bemestingsstrategie resulteerde in vergelijkbare opbrengsten vergeleken met een gangbaar systeem. Deze stapsgewijze verhoging van de N-belasting veroorzaakte beperkte hoeveelheden vrij ammoniak en ammonium die mogelijke plantintoxicatie kan veroorzaken.

Tenslotte in [Hoofdstuk 6] vergeleken we in één groeijaar (februari-november) durende tomatenteelt onderzoek de chemische karakteristieken en de microbiële gemeenschap in vier tomatenteeltsystemen (grond versus grondloos en organische versus minerale bemesting) evenals de groei en tomatenopbrengst. Phospholipid fatty acid profiling (PLFA) werd gebruikt om de complexe microbiële interactie te ontrafelen. We toonden aan dat in de vier verschillende teeltsystemen de samenstelling van de microbiële gemeenschap verschilde. De grondloze teelt gecombineerd met de organische bemesting vertoonde een opbrengstverschil van 15% in vergelijking met de minerale controle. Het gebruik van organische meststof in combinatie met een organisch substraat is een beloftevolle benadering in de richting van een duurzame tuinbouw. Bijkomend onderzoek is nodig om de resultaten van dit onderzoek verder te bevestigen.

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CURRICULUM VITAE

Personal Information

Oliver Grunert

Married with Katrien van Melckebeke

Children: Florian (11/6/2003) and Niels Grunert (24/2/2005)

Born: March 19th 1973, Opladen-Leverkusen, Germany

Nationality: Belgian - German

Zeugnis der allgemeinen Hochschulreife (Abitur) in 1992 in Fulda (Germany)

German army: 15 months (1992-1993)

Contact information:

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9831 Deurle

Belgium

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Education

PhD in Applied Biological Sciences 2012 – present

Center for Microbial Ecology and technology (CMET), Ghent University – Belgium

Promotors: prof. dr. ir. Nico Boon, prof. dr. ir. Dirk Reheul, prof. dr.ir. Marie-Christine Van Labeke

Topic: *Microbially managed organic growing media for horticulture*

Funding: personal scholarship IWT-Baekeland (120200)

M.Sc.: Bio-ir cell- and gene biotechnology 1993-1998

Faculty of Applied Engineering Sciences, Ghent University

Graduated with distinction

Master thesis: *Exchange of catabolic plasmids between bacteria in activated sludge and its importance for bio-augmentation (Prof. Dr. ir. W. Verstraete)*

Working experience and acquired skills:

PhD researcher 2012 – present

Laboratory of Microbiology and Technology (LabMET), Ghent University

Expertise in the field of environmental biotechnology and horticulture

- 3 international peer reviewed publications

- several oral presentations at international conferences

Coaching and teaching skills

Tutor of 4 master thesis

Tutor of 5 bachelor students

Assistant of practical exercises at LabMET/CMET

Courses (doctoral schools)

Opportunity recognition workshop – iMinds (2-4 July 2014)

Advanced Academic English

Theory and Methods of Research in Applied Biological Sciences (27-31 May 2013)

Effective scientific communication

Effective slide design

Personal effectiveness

Plunge in your own business plan (Food2Know)

Introductory statistics. Basics of statistical Interference

Introduction to R.

Analysis of Variance

Applied Longitudinal Analysis

Arctic Microbiology: education and training in field work and analysis. Iceland

Professional working experience

Greenyard/Peltracom *2003- until now*

Quality assistant, R&D project manager, Internal auditor quality according ISO 19011:2011"

Teeltinfo cvba *2002-2003*

Horticultural advisory service in Belgium, the Netherlands and Poland

Departement Crop Husbandry and Ecophysiology *1999 – 2002*

CLO-Gent research assistent of Prof. dr. h.c. dr. ir. L. Carlier

Summary of contributing projects :

European ManureEcoMine Project:

WP5 leader of the project: Green fertilizer upcycling from manure: Technological, economic and environmental sustainability demonstration with Grant agreement no. 603744

European project Urban Loops:

WP leader concerning fertilizer blending (1st round)

MIP project's:

MicroNOD, I-LOVE-T, Heat4Peat, DUPOCO

Acquired grants

Grant (Grasslands Young Researchers in Europe) subsidized by the European Union for EGF 2002 in La Rochelle : Multi-Functional Grasslands Quality Forages, Animal Products and Landscapes

Publications

A1 - accepted

Grunert, Oliver, et al. "Mineral and organic growing media have distinct community structure, stability and functionality in soilless culture systems."*Scientific reports* 6 (2016).

Coppens, Joeri, et al. "The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels." *Journal of Applied Phycology* (2015): 1-11.

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A4

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Grunert, O. en Van Waes, J. 2002. Koudetolerantie bij maisrassen. Landbouwleven, 1 maart 2002

C1: congres proceedings

Grunert, O., Hernandez-Sanabria, E., Perneel, M., Van Labeke, M. C., Reheul, D., & Boon, N. (2014). Molecular insights on the functional microbial community from organic and mineral growing media and its interaction with agrobacterium rhizogenes. Communications in agricultural and applied biological sciences, 79(3), 345.

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O. Grunert, M. Perneel, and S. Vandaele. New developments in organic grow bags based on peat as an answer on the actual waste problems of rock wool. Peltracom NV, Scheepzatestraat 50, Kade 900, 9000 Gent. email: R&D@peltracom.be. http://www.pole-tourbieres.org/docs/Lamoura_Grunert.pdf.

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C2: Patents

Improved grow bag for the cultivation of crops: WIPO Patent Application WO/2008/006181: Improved grow bag for cultivating crops characterized in that it mainly consists of a compostable bag in which has been provided a predominantly organic substrate.

Poster Presentations

A.A. Robles Aguilar, O. Grunert, J. Postma, S.D. Schrey, V. Temperton, S. Blossfeld, E. Hernandez-Sanabria, D. Reheul, N. Boon, N.D. Jablonowski. Response of tomato and narrow-leaved lupin root system architecture and rhizosphere dynamics to nitrogen source. EcoSummit 2016, Ecological Sustainability: Engineering Change, Montpellier (FR). 29/8/2016-1/9/2016

O. Grunert, E. Hernandez-Sanabria, MC. Van Labeke, D. Reheul, M. Perneel, N. Boon. Microbial community dynamics of growing media in soilless culture systems. Maastricht, The Netherlands. 21-25 June 2015.

A. A. Robles Aguilar, O. Grunert, N. D. Jablonowksi, N. Boon, V. Temperton, S. Blossfeld, E. Hernandez-Sanabria, D. Reheul. Dynamics of the microbial community structure in the rhizosphere of narrowleafed lupin and tomato as related to nitrogen form provided. Maastricht, The Netherlands. 21-25 June 2015.

A. De Vliegher, O. Grunert and L. Carlier (2003). Cutting or grazing in autumn: effect on grass yield, grass quality and soil nitrate content. EGF 2003 in Pleven. (May 2003).

O. Grunert, J. Van Waes, D. Reheul and L. Carlier (2002). Cold tolerance of Zea mays L. : A tentative field experiment. 8th PhD Symposium on Applied Biological Sciences. Het Pand, Gent , 9 October 2002.

A. De Vliegher, O. Grunert and L. Carlier (2002). The effect of graslandresowing on yield and quality under grazing conditions. EGF 2002 in La Rochelle : Multi-Functional Grasslands Quality Forages, Animal Products and Landscapes

O. Grunert, A. De Vliegher and L. Carlier (2002). Residual mineral nitrogen in the soil on grassland. EGF 2002 in La Rochelle : Multi-Functional Grasslands Quality Forages, Animal Products and Landscapes.

A. De Vliegher, O. Grunert and L. Carlier (2000). Yield of renovated grassland under grazing conditions. EGF 2000 in Aalborg Denmark (20 - 25 May 2000)

<u>Oral Presentations</u>

O. Grunert, J. Coppens, S. Van Den Hende, I. Vanhoutte, N. Boon, L. De Gelder, G. Haesaert. Tomatoes, trends towards 2020. The application of microalgae as a slow-release fertilizer: tomato cultivation as a model. Antwerp. April 13-15, 2016.

O. Grunert, E. Hernandez-Sanabria, N. Ameloot, T. Beyers, S. De Neve, M.-C. Van Labeke, D. Reheul, and N. Boon. Four sustainable tomato cultivating systems of the future: comparison of the microbial community in relation to tomato growth. Tomatoes, trends towards 2020. Antwerp. April 13-15, 2016.

O. Grunert et al. struvite and organic Fertilizer Impacting The Rhizosphere Microbial Community, Nutrient Turnover and Tomato Plant Growth Performance. WEF/IWA Nutrient Removal and Recovery 2016. Denver (USA)

O. Grunert, A. A. Robles Aguilar, E. Hernandez-Sanabria, D. Reheul, N. Boon and N. D. Jablonwoski . fertilizer type influences dynamics of the microbial community structure in the rhizosphere of tomato and impact the nutrient turnover and plant performance. National Symposium for Applied Biological Sciences in Antwerp. 5th of February 2016

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O. Grunert. High-throughput sequencing analysis provides a comprehensive insight into the complex bacterial relationships in horticultural substrates. Susgro 2015. International symposium on Growing media, composting and substrate Analysis. 7-11 Sept 2015.

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COST Action 814. Crop development for the cool and wet regions of Europe: Workshop on N-use efficiency. 2 – 5 June 1999, Melle, Belgium: O. Grunert, I. Verbruggen, L. Carlier and Van Bockstaele (1999). Reduced nitrogen fertilization on grasses and forages.

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DANKWOORD

Waarom ben je hier aan begonnen? Deze vraag heeft men mij vele keren gesteld en ik heb mijzelf ook vaak deze vraag gesteld. Is het een mid-life crisis op je 40ste? Moeilijk om hier een antwoord op te geven, maar het was in elk geval sterker dan mezelf. Ik heb een zekere rusteloosheid in mij, een drang naar kennis en een drang om grenzen te verleggen, de behoefte om af te zien en iets nieuws te ontdekken. Neen, Katrien, je hoeft je geen zorgen te maken. Ik ben maar een gewone rakker, die zich graag in de tuin bezig houdt en graag eens de spade in de grond steekt op zoek naar wat verzet. Ik weet dat deze periode niet enkel van mij veel gevraagd heeft, maar ook van iedereen in mijn onmiddellijke en minder onmiddellijke omgeving. Sorry hiervoor, maar ook een dikke merci omdat ik de kans gekregen heb dit te mogen doen. Een ding weet ik zeker, dit doctoraat was in elk geval een "Total-Erlebnis", het was allesomvattend.

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