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BIOCHAR IN PERENNIAL CROPS: NUTRITIONAL, AGRONOMICAL AND ENVIRONMENTAL IMPLICATIONS

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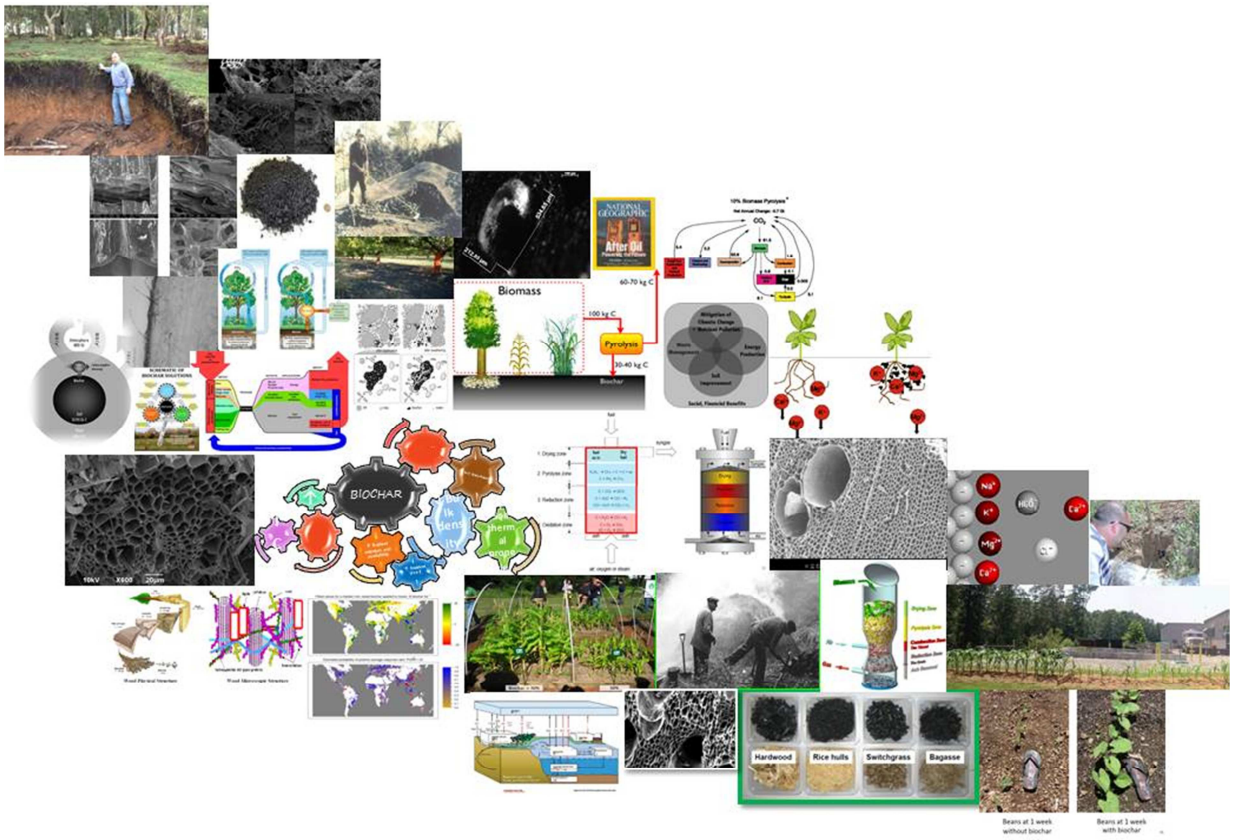

Giovambattista Sorrenti

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March, 2015

.....to the Mother Earth, the most amazing place where living, the only one we have.....

"A nation that destroys its soil, destroys itself"

Franklin Delano Roosevelt

February, 26 1937

To my parents

Alessandro and Esterina

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BIOCHAR IN PERENNIAL CROPS: NUTRITIONAL, AGRONOMICAL AND ENVIRONMENTAL IMPLICATIONS

ABSTRACT

Biochar is the solid C-rich matrix obtained by pyrolysis of biomasses, currently promoted as a soil amendment with the aim to offset anthropogenic C emissions, while ameliorating soil properties and growth conditions. Benefits from biochar seem promising, although scientific understandings are beginning to be explored. In this project, I performed a suite of experiments in controlled and in field conditions with the aims to investigate the effect of biochar on: a) the interaction with minerals; b) Fe nutrition in kiwifruit; c) soil leaching, soil fertility, soil CO_2 emissions partitioning, soil bacterial profile and key gene expression of soil nitrification-involved bacteria; d) plant growth, nutritional status, yield, fruit quality and e) its physical-chemical changes as affected by long-term environmental exposure. Biochar released K, P and Mg but retained Fe, Mn, Cu and Zn on its surface which in turn hindered Fe nutrition of kiwifruit trees. A redox reaction on the biochar surface exposed to a Fe source was elucidated. Biochar reduced the amount of leached NH_4^+-N but increased that of Hg, K, P, Mo, Se and Sn. Furthermore, biochar synergistically interacted with compost increasing soil field capacity, fertility, leaching of DOC, TDN and R_{SOC}, suggesting a priming effect. However, in field conditions, biochar did not affect yield, nutritional status and fruit quality. *Actinomadura flavalba*, *Saccharomonospora viridis*, *Thermosporomyces composti* and *Enterobacter* spp. were peculiar of the soil amended with biochar plus compost which exhibited the highest band richness and promoted gene expression levels of *Nitrosomonas* spp., *Nitrobacter* spp. and enzymatic-related activity. Environmental exposure reduced C, K, pH and water infiltration of biochar which instead resulted in a higher O, Si, N, Na, Al, Ca, Mn and Fe at%. Oxidation occurred on the aged biochar surface, it decreased progressively with depth and induced the development of O-containing functional groups, up to 75nm depth.

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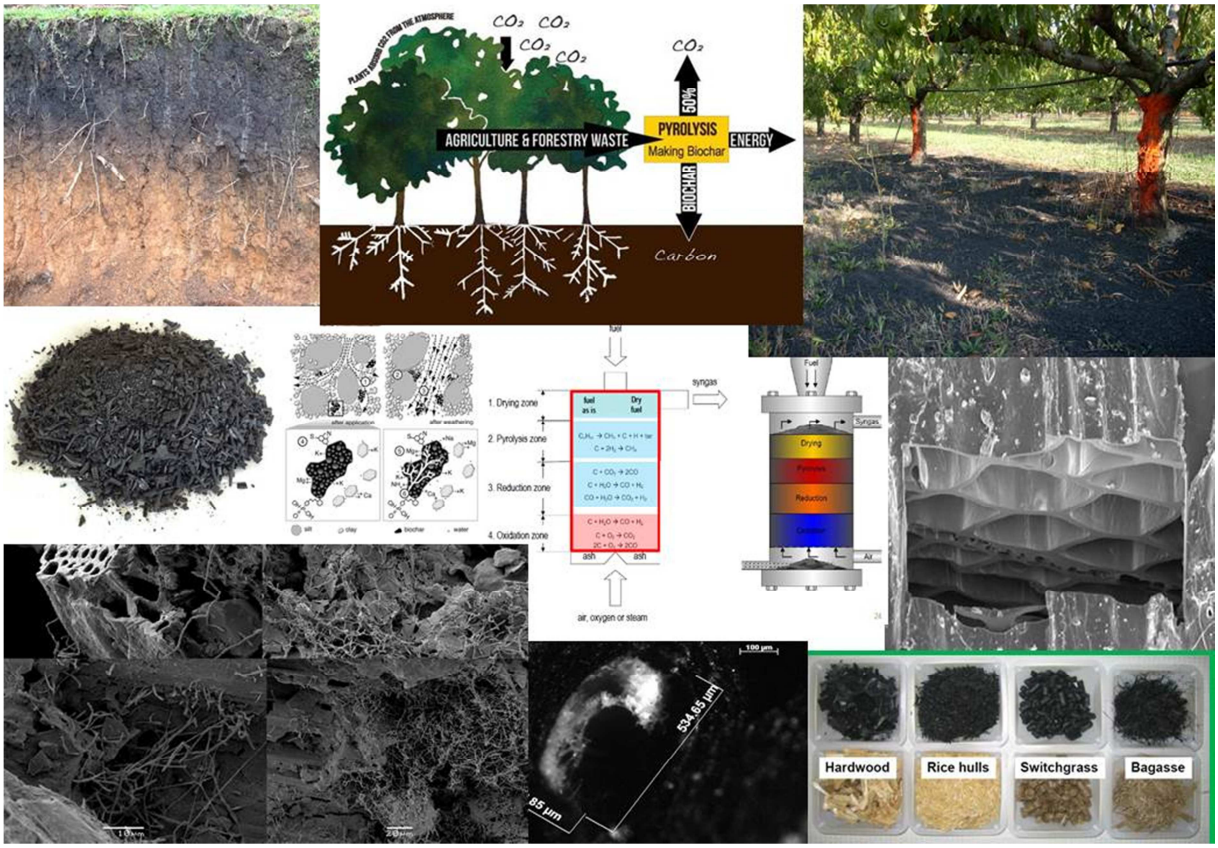
PLANTS ABSORB CO2 FROM THE ATMOSPHERE
CO2
AGRICULTURE & FORESTRY WASTE
PYROLYSIS
Making Biochar
ENERGY
BIOCHAR
50%
Carbon
1. Drying zone
2. Pyrolysis zone
3. Reduction zone
4. Oxidation zone
fuel as is
Dry fuel
ash
syngas
air, oxygen or steam
Fuel
Syngas
Drying
Pyrolysis
Reduction
Oxidation
Air
100 µm
534.65 µm
85 µm
Hardwood
Rice hulls
Switchgrass
Bagasse

LIST OF ABBREVIATIONS and ACRONYMS

AAS	Atomic Absorption Spectrophotometry
AFOLU	Agriculture, Forestry and Other Land Use
AEC	Anion Exchange Capacity
ANOVA	Analysis of Variance
AVG	Average
AWC	Available Water Content
BNF	Biological Nitrogen Fixation
CE	Electrical Conductivity
Chl	Chlorophyll
CO_2 eq	Carbon Dioxide Equivalent
CEC	Cation Exchange Capacity
CFU	Colony Forming Unit
DGGE	Denaturing Gradient Gel Electrophoresis
DIN	Dissolved Inorganic Nitrogen
DL	Detection Limit
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
DTPA	Diethylenetriamine pentaacetic acid
DW	Dry Weight
Ea.	Each
EC	Electrical Conductivity
EDS	Energy Dispersive Spectroscopy
ETo	Evapotranspiration
FC	Field Capacity
FW	Fresh Weight
GHGs	Greenhouse Gases
ICP	Inductive Coupled Plasma
ID	Internal Diameter
Kc	Crop Coefficient
M	Molarity
MCLG	Maximum Contaminant Level Goal
MF	Mycorrhizal Fungi
MPN	Most Probable Number
NPP	Net Primary Production
NMR	Nuclear Magnetic Resonance
OM	Organic Matter
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
POPs	Persistent Organic Pollution
PCDD	Polychlorinated Dibenzo-p-dioxins
PCDF	Polychlorinated Dibenzofurans
R_R	Rhizosphere Respiration
R_{SOC}	Soil Organic C-derived Respiration
R_{TOT}	Total Soil Respiration
SA	Surface Area
SE	Standard Error
SEM	Standard Error of Means
SEM	Scanning Electron Microscopy

SLW	Specific Leaf Weight
SNK	Student Newman Keuls
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SSC	Soluble Solid Content
SWC	Soil Water Content
T	Temperature
TA	Titratable Acidity
TDN	Total Dissolved Nitrogen
TCN	Total Combustible Nitrogen
TEM	Transmission Electron Microscopy
TOC	Total Organic Carbon
UR	Relative Humidity
XPS	X-Ray Photoelectron Spectroscopy
WHC	Water Holding Capacity
φ	Porosity
ρe (ED)	Envelope Density
ρs (SD)	Skeletal Density

LIST OF UNITS

Bar	1 bar = 100 kPa =0.987 atm
°C (sec^{-1}) (min^{-1})	Degree Celsius (per second) (per minute)
cm	Centimeters
Cmolc g^{-1}	Centimol of charge (1 cmolc kg^{-1} = meq 100 g^{-1}) per gram
µS (dS) cm^{-1}	Microsiemens (10^{-6} siemens) (Decisiemens) per centimeter
eV	Electronvolt=1.602176565(35)×10^{-19} J
g CO_2 m^{-2} h^{-1}	Grams of C dioxide per square meter per hour
g (kg^{-1}) (cm^{-3})	Grams (per kilogram) (per cubic centimeter)
g ha^{-1} ($year^{-1}$)	Grams per hectare (per year)
Gt (y^{-1})	Gigatonnes (per year)
h	Hours
ha (y^{-1})	Hectares (per year)
J g^{-1} K^{-1}	Joule (1J= 1 kg m^2 sec^{-1}) per gram per Kelvin
K	Kelvin (1K= °C+273.15)
Kg (ha^{-1}) ($year^{-1}$)	Kilograms (10^3 grams) (per hectare) (per year)
Km^2	Square kilometers
kJ mol^{-1}	Kilojoule (10^3 J) per mole
kPa	Kilo Pascal (10^3 Pa= 10^{-2} bar)
L (h^{-1})	Liters (per hour)
Lx	Lux 1 lx = 1 lumen per square meter
m - m^2 - m^3	Meters - Square meters - Cubic meters
meq (g^{-1})	Milliequivalents (per gram)
min	Minutes
mg (L^{-1}) (kg^{-1})	Milligrams (10^{-3} g) (per liter) (per kilos)
Mg ha^{-1} (m^{-3})	Megagrams (10^6 g) per hectare (per cubic meter)
mL	Milliliters
mm	Millimeters
mm^3 (g^{-1})	Cubic millimeters (per gram)
MPa	Mega Pascal
mS cm^{-1}	Millisiemens per centimeter
Mt	Millions of tons
nm (ng)	Nanometers (10^{-9} meters) Nanograms (10^{-9} grams)
rpm	Revolution per Minute
Pg	Petagrams (10^9 tonnes)
ppm	part per million
Pt	Petatonnes (10^{15} tonnes)
Sec	Seconds
t (ha^{-1}) (m^{-3})	Tonnes (per hectare) (per cubic meter)
V (mV)	Volts (Millivolts)
v v^{-1}	Volume per volume
W	Watt (1 kg·m^2 s^{-3})
w w^{-1} (w v^{-1})	Weight per weight (weight per volume)
µg	Micrograms (10^{-6} grams)
µL	Microliters (10^{-6} liters)
µm	Micrometers (10^{-6} meters)
% (y^{-1})	Percent (per year)
%at	Relative surface atomic composition
‰	Parts per mille

LIST OF CHEMICAL ELEMENTS AND FORMULAS

Al	Aluminum	**Mg**	Magnesium
Ag	Silver	**$MgCl_2$**	Magnesium chloride
Ar	Argon	**Mn**	Manganese
As	Arsenic	**$MnCl_2$**	Manganese chloride
B	Boron	**Mo**	Molybdenum
Ba	Barium	**N**	Nitrogen
Be	Beryllium	**N_2**	Nitrogen (gaseous form)
C	Carbon	**N_2O**	Nitrous oxide
Ca	Calcium	**Na**	Sodium
$CaCl_2$	Calcium chloride	**Na_2O**	Sodium oxide
$CaCO_3$	Calcium carbonate	**NaOH**	Sodium hydroxide
CaO	Calcium oxide	**NH_3^+**	Ammonia
Cd	Cadmium	**NH_4^+**	Ammonium (ion)
CH_4	Methane	**NH_4Cl**	Ammonium chloride
Cl	Chlorine	**NH_4^+-N**	Ammonium nitrogen
Co	Cobalt	**NH_4NO_3**	Ammonium nitrate
CO	Carbon oxide	**Ni**	Nickel
CO_2	Carbon dioxide	**NO_x**	Mono-nitrogen oxides
Cr	Chromium	**NO_3^-**	Nitrate (ion)
CsCl	Cesium chloride	**NO_3^--N**	Nitrate nitrogen
Cu	Copper	**O (O_2)**	Oxygen
Fe	Iron	**P**	Phosphorus
$FeCl_3$	Iron chloride	**Pb**	Lead
$FeCO_3$	Iron carbonate	**PO_3^{--}**	Phosphate
$FeSO_4$	Fe(II) sulphate	**P_2O_5**	Phosphorus pentoxide
H (H_2)	Hydrogen (gas)	**S**	Sulphur
H_2O (d-H_2O)	Water (Deionized water)	**Sb**	Antimony
H_2SO_4	Sulphuric acid	**Se**	Selenium
H_3PO_3	Phosphoric acid	**Si**	Silicon
HNO_3	Nitric acid	**Sn**	Tin
HCl	Hydrochloric acid	**SiO_2**	Silica (silicon dioxide)
He	Helium	**SO_3**	Sulfur trioxide
Hg	Mercury	**$SO_4(NH_4)_2$**	Ammonium sulphate
K	Potassium	**Sr**	Strontium
KCl	Potassium chloride	**Ti**	Titanium
KNO_3	Potassium nitrate	**Tl**	Thallium
K_2O	Potassium oxide	**V**	Vanadium
K_2CO_3	Potassium carbonate	**Zn**	Zinc
$LaCl_3$	Lanthanum chloride	**$ZnCl_2$**	Zinc chloride
Li	Lithium		

CHAPTER 1

INTRODUCTION

Anthropogenic carbon dioxide (CO_2) emissions, mainly from fossil fuel consumption, have increased in the last decade at a rate of over 0.9 Gt y^{-1}, corresponding to 3% of the net global emissions (Woolf et al., 2010) and reached the record of 31.6 Gt CO_2 eq y^{-1} in 2012, the highest level in history (IEA, 2013), contributing to worsen the "greenhouse effect", so the climate changes (Lehmann, 2007a). Solomon et al. (2009) estimated an increase of about 1 mg kg^{-1} in the atmospheric CO_2 content each 4 Gt of fossil carbon (C) burning and worldwide scientists point out that the increased CO_2 concentration in the atmosphere (which shifted from 280 ppm measured in the pre-industrial age to the current CO_2eq concentration of 430 ppm) represents, together with others greenhouse gases (GHGs), such as nitrous oxide (N_2O) and methane (CH_4), the major driver for permanent global climate changes. Estimations indicate that if global GHGs emission will be not offset, their concentration in the atmosphere could triplicate the pre-industrial level within 2100, causing an overall warming effect up to 6.4°C (IEA, 2013). A recent survey confirmed that agriculture represents the largest contributor to non-CO_2 GHGs emission and that over the past 20 years, GHGs net emission from AFOLU (Agriculture, Forestry and Other Land Use) increased by 8%, from an average of 7,497 Mt CO_2eq in the 1990s to an average of 8,103 Mt CO_2 eq in the 2010s (FAO, 2014).

Concerns due to climate changes may include land degradation, loss of biodiversity, increase of coastal areas vulnerability and alterations in land hydrology (Ravishankara et al., 2009; Solomon et al., 2009) with serious implications for world food security, human economy, biodiversity and potentially affecting the more vulnerable socio-economic segments first (Lal, 2010a; 2010b; Ericksen et al., 2009). In fact, according to Ernsting (2011), the global warming will particularly affect poor societies living in developing Countries first whereas developed Countries are still the major drivers.

Therefore, global GHGs emission must be worldwide urgently cut down through the development of effective and sustainable strategies in order to withdraw at least 3.2 Gt of CO_2 eq y^{-1} from the atmosphere (Sohi, 2012). Other than reducing global energy consumption, raising the energetic efficiency, decreasing the emissions of concrete building and replacing fossil fuel energy with alternative and renewable GHGs neutral energy, either plant biomass or soil organic matter (SOM) are considered valuable strategies to both sequester and store C from the atmosphere (Lackner, 2003).

Photosintetically fixed C (net primary production, NPP) represents a substantial C flow which consists of about 57 Gt y^{-1} and 50 Gt y^{-1} in terrestrial and oceanic ecosystems, respectively (Behrenfeld et al., 2001). A significant portion of the global NPP could be destined to produce renewable energy, thereby contributing to offsets GHGs emission. Similarly, most of the agricultural residues and organic wastes that currently are mainly buried in soil, disposed and/or open burnt, represent a valuable source to yield renewable energy. However, either when biomass naturally decomposes or it is used as a fuel to produce other sources of energy (e.g. electricity, gases), fixed C returns into the atmosphere. Likewise, most of the amount of organic amendments applied to agricultural soils in order to increase the SOM content is often quickly depleted since C is used as substrate by edaphic microorganisms to fulfill their energy requirements.

In this scenario, the C balance of such strategies is neutral (Lehmann, 2007b), hence their long-term C sequestration potential negligible.

Conversely, C becomes more steadily fixed when organic materials are charred, so that the conversion of biomasses into charcoal and its subsequent use as a soil conditioner (biochar) has been recently proposed as a sustainable long-term strategy to sequester atmospheric CO_2, whilst potentially mitigating the global climate change (Stavi and Lal, 2013; Sohi, 2012; Shrestha et al., 2010; Woolf et al., 2010; Lehmann and Joseph, 2009; Gaunt and Cowie 2009; Laird, 2008, Lehmann, 2007a).

Biochar is defined as a black, fine-grained, highly porous and recalcitrant C-rich material generated by the pyrolysis of biomasses in oxygen-limited conditions (Lehmann et al., 2006; Lehmann, 2007a) (Fig. 1.1). The intended purpose distinguishes biochar from the common charcoal which is mainly adopted as a fuel, a filter, a reductant agent in iron-making or a coloring agent in industry (Lehmann and Joseph, 2009). More specifically, biochar is produced by the thermal decomposition (incomplete combustion) of organic materials (such as wood, organic wastes, agro-industrial residues, energetic crops, manures, municipal organic solid wastes) under limited supply of oxygen (O_2) and at relatively low temperatures (<700°C) (Hammes et al., 2008). Ultimately, biochar is the by-product of the pyrolysis process which has a high C content and aromatic compounds whit 6 C atom rings linked together either with O and/or hydrogen (H), the most abundant atoms in organic matter (OM) (Lehmann and Joseph, 2009).

Figure 1.1. Biochar from hardwood pyrolized at 500 °C
(Picture: Sorrenti, 2013)

1.1 Biochar: terminology and history

The term biochar was first adopted in 1998, then widely used from 2006 to indicate the manmade charcoal produced for agronomical purposes (Lehmann et al., 2006) and, in particular, as a soil amendment since it has been observed that charcoal, other than sequestering C, improves physical, chemical and biological properties of amended soils (Baronti et al., 2014; Spokas et al., 2012; Verheijen et al., 2010; Laird, 2008; Steiner et al., 2007; Lehmann et al., 2003). In fact, it has been reported that biochar application to soils induces many advantages including enhancement of soil health (Ameloot et al., 2013), reduction of heavy metal contamination risks (Namgay et al., 2010), increase of plant growth and yield (Jeffery et al., 2011; Major et al., 2010; Chan et al., 2008;) and decrease of GHGs emissions from soil (Singh et al., 2010a; Van Zwieten et al., 2009; Yanai et al., 2007).

However, biochar doesn't represent a new discover since its potential as a soil conditioner was well known centuries ago in the Amazon basin where soils (Oxisols, Anthrosols) are typically acid, dark red-colored, rich in aluminum (Al), manganese (Mn) and iron (Fe) and characterized by high mineralization rates, that makes fertility poor (Glaser et al., 2002; 2001). In this region, only limited spots, called "Terra Preta de Indio" are characterized by alkaline, dark-colored and

fertile soils (Fig. 1.2) (Glaser and Birk, 2012; Sombroek et al., 2003). Terra Preta de Indio sites are found as small areas of about 200 m in diameter each, usually close to both current and historic human settlements throughout Amazonia, covering a total area of about 18,000 km^2 (Sombroek and Carvalho de Souza, 2000) with varying depth (down to 1 m).

Figure 1.2. "Terra Preta" soil profile (Brazil, 2013). The dark color of the first 0.50 m depth is due to anthropogenic C.
(Pictures: Sorrenti, 2013)

Terra Preta de Indio soils were obtained between 2000 and 1500 years ago by the common activities of the local Amazon Indians who, for centuries, enriched their fields with charcoal mixed with different organic sources (e.g. fish and animal bones, plants, organic wastes, potsherds and feces) (Mann, 2002). Terra Preta de Indio soils contain up to 250 Mg ha^{-1} of soil organic carbon (SOC) in the top 0.30 m (compared to 100 Mg ha^{-1} of surrounding soils) and up to 500 Mg ha^{-1} in the top 1m (Glaser, 2001), is richer in nutrients (e.g. nitrogen (N), sulphur (S), calcium (Ca) and phosphorus (P)), retains more water, has a higher microbial activity, higher pH and cation exchange capacity (CEC), reduces nutrient leaching and increases microbial diversity (Glaser and Birk, 2012; O'Neill et al., 2009; Kim et al., 2007), therefore is considered more fertile than surrounding Oxisols (Glaser et al., 2001; Mann, 2002). For these reasons, it has been reported that biochar has positive effects on crop yields in Oxisols as well as in other tropical soils (Lehmann and Joseph, 2009).

Soil types comparable with those found in the Amazon basin have been discovered, although in smaller scale, in Amazon parts of Peru, Columbia and southern Venezuela and in the Guianas (Heckenberger et al., 2003; Kern et al., 2003; Sombroek et al., 2002; Denevan, 1996) in Sierra Leone (Africa), Liberia (Africa) and Kalimantan (Indonesia). The fact that black soils are most diffused and larger in the Amazon basin seems to be related to the differences in the

technology available to the local populations at that time. In fact, only the Indians of South America ignored Fe yet (then no machetes or other Fe-made tools were available to cut vegetation) hence, in order to exploit the lands, trees (and biomasses) were burned *in situ* then covered with soil while still burning (limiting the O), involuntary originating charcoal.

These findings have recently attracted the interest of scientists in developing the so called "Terra Preta Nova" soils which would mimic the "Terra Preta de Indio" soils by the incorporation of pyrolized biomasses in order to improve soil functions while mitigating climate change by sequestering C (Ameloot et al., 2013).

1.2 Pyrolysis of biomasses

Pyrolysis is defined as the thermo-chemical decomposition of any organic material by heating in the absence (or limited availability) of O, although a small amount of oxidation will always occur (Laird et al., 2009). The term derives from two Greek worlds where "pyro" and "lysis" mean fire and decomposition, respectively and the fundamental aim of this process is to transform a solid ash-rich feedstock into bio-oil which is an ash-poor liquid product. Compared to combustion during which total oxidation of OM occurs, the degree of oxidation observed with pyrolysis is much smaller leading to a substantially larger proportion of C in the charred material which is not liberate in the atmosphere as CO_2. Pyrolysis may occur spontaneously at specific temperature varying with material (e.g. 300°C for wood) and in nature it occurs when vegetation is exposed to wildfires.

Pyrolysis degrades feedstock polymers into smaller compounds while larger molecules are also produced (including both aromatic and aliphatic compounds) as a consequence of polymerization of OM. Nevertheless, with pyrolysis much of the C from the feedstock is converted into three different components such as gases, liquids and solids in different proportions (Tab 1.1) depending upon both the feedstock and the pyrolysis conditions used (Laird et al., 2009).

Produced syngas include both flammable CH_4 and other hydrocarbons which can be condensed by cooling, leading to oil and tar residues. Products of pyrolysis, either gas (condensed or in gaseous forms) or liquids can be used as a fuel for combustion while biochar represents the byproduct.

The first evidence for charcoal production by pyrolysis comes from Southern Europe and the Middle East, more than 5500 years ago. Subsequently, with the coming Bronze Age (4000 years ago), pyrolysis was widely adopted since it was essential to produce charcoal in order to sustain the fire needed to produce bronze by smelting tin (Sn) added with copper (Cu).

Although the basic concept of pyrolysis remains unchanged, modern technologies are adopted, each one producing different percentage of outputs. Pyrolysis can be manipulated, apart from feedstocks, by the temperature (rates and peaks) and residence time of the feedstock in the reactor unit. Either temperature or residence times of solid or vapor in the pyrolysis unit, or a combination of both, have a large effect on the relative proportions of the end products. Because of it, four different types of pyrolysis are generally identified: i) fast, ii) intermediate, iii) slow pyrolysis and iv) gasification (due to the high proportion of syngas produced) (Tab. 1.1).

Table 1.1. Product yield of bio-oil, syngas and biochar under different pyrolysis conditions

Process	**T °C**	**Exposure time**	**Yield (%)**		
			Liquid (Bio-oil)	***Gases (Syngas)***	***Solid (Biochar)***
Fast pyrolysis	~500	< 2 sec.	75	13	12
Intermediate pyrolysis	350-450	10-20 sec.	50	30	20
Slow pyrolysis	300-400	10-30 min.	30	35	35
Gasification	>750	2-4 min.	5	85	10

Source: modified from IEA, 2007

1.3 The sustainable-biochar concept: a C negative strategy to mitigate climate change

As mentioned, pyrolysis represents a sustainable strategy for producing renewable energy since it thermally transforms biomasses, organic wastes and biorefining residuals into bio-oil, syngas and biochar (Laird, 2008). Due to its high recalcitrance, biochar serves as a C sink since it persists up to thousands of years when incorporated into the soils (Spokas et al., 2012; Verheijen et al., 2010; Lehmann, 2007a): in other words, biochar significantly reduces the rate at which photosynthetically fixed C compounds return to the atmosphere with a positive C removal from atmosphere (Sohi, 2012; Lal, 2010b; Shackley et al., 2010; Lehmann and Joseph, 2009). Recently, it has been estimated that an extensive use of this strategy in agriculture can offset current global C emissions by a maximum of 1.8 Gt CO_2-C eq., corresponding to the 12% y^{-1} of anthropogenic CO_2-C and reducing net emissions over a century by 130 Gt CO_2-C, without compromising food security and soil conservation (Woolf et al., 2010). Biochar can be conveniently produced by large industrial plants down to small domestic scale level exploiting either several commercial or homemade pyrolysis units able to yield different proportions of biochar, bio-oil and syngas (Tab. 1.1). Then, syngas is typically intended to generate electricity

while the bio-oil may be used for heating applications and, after adequate treatments, potentially used as a biodiesel substitute.

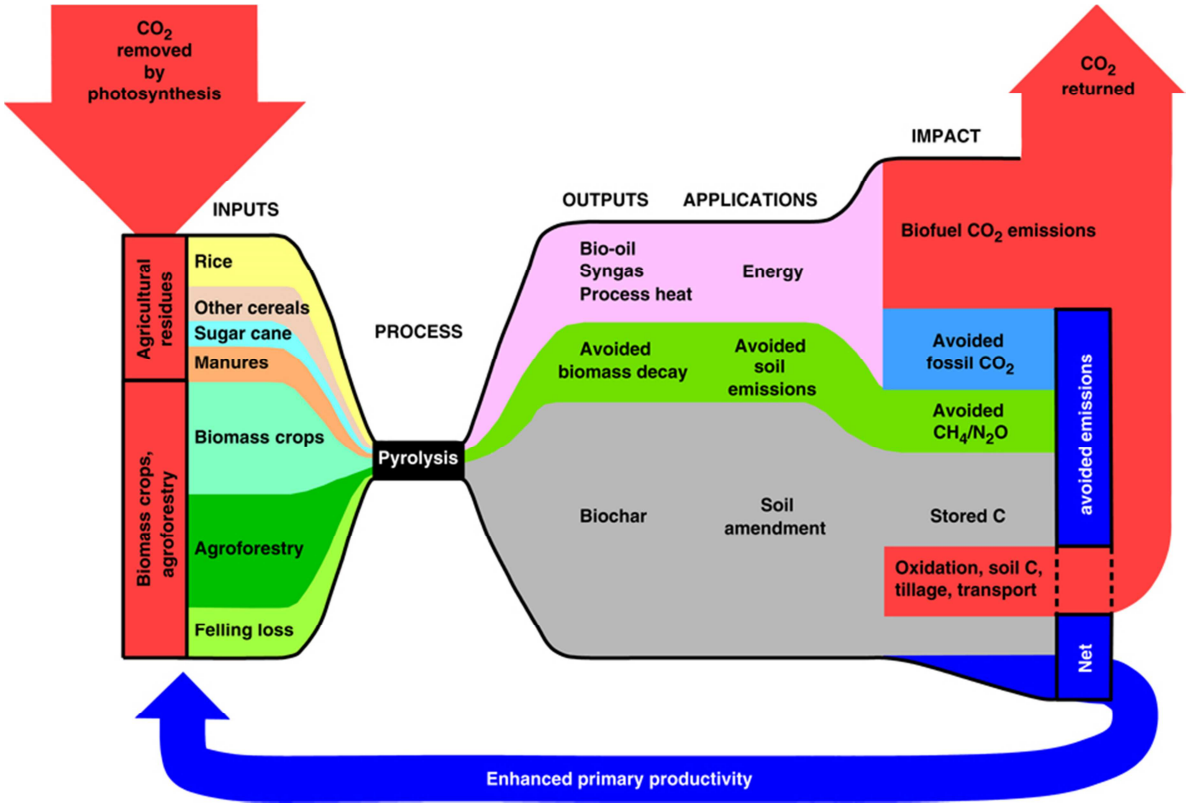

Figure 1.3. The sustainable biochar concept. Size of each component is proportional to its significance (source-effect).

Source: Woolf et al., 2010. Nature communications, 1, 56 - (with permission)

The sustainable biochar concept, resumed in Fig. 1.3, starts removing CO_2 from the atmosphere by plants which convert it, by photosynthesis, into biomass. Biomass undergoes the pyrolysis process to yield renewable energy sources (syngas and bio-oil) and, as a by-product, biochar. The bioenergy produced by pyrolysis can offset fossil C emissions which would be inevitable to produce the same amount of energy exploiting non-renewable sources (e.g. fossil coal). Besides, if incorporated into the soil, biochar stores C much longer than would occur if the biomass would have been left to naturally or artificially degrade. Also, avoiding biomass decay, emissions of CH_4 and N_2O are prevented. Additionally, benefits induced by biochar to soil fertility may increase plant NPP with further removal of atmospheric CO_2 by photosynthesis, thereby achieving a positive feedback.

1.4 Biochar stability

Biochar is considered a recalcitrant C-rich material (Nguyen and Lehmann, 2009) as a consequence of its predominantly condensed aromatic structure (Baldock and Smernik, 2002) and in comparison to other organic materials, biochar resists to microbial and/or natural environmental decomposition (Lehmann et al., 2007a). However, not all biochars have the same stability in soil, depending on the biomass used and the pyrolysis conditions. Generally, biochars account for both labile and stable forms of C. The latter (highly aromatic) is believed to be stable in soils likely for several thousand years whereas the labile C forms (low aromacity) can be quickly degraded and released to the atmosphere as CO_2 within a couple of years after its incorporation. The labile C fraction in biochar ranges from 2 to 12 % whit the highest rate obtained when low heating rates (slow pyrolysis) are adopted. In fact, above 475 °C carbohydrates are almost entirely converted to volatiles then, remaining C compounds are highly aromatic and stables, whereas partially unconverted biomass mineralizes quickly once in the soil (Yang et al., 2007).

Several studies report soil residence time of either charcoal or biochars in timescales ranging from decades to millennia (Zimmerman, 2010; Kuzyakov et al., 2009; Cheng et al., 2008; Hammes et al., 2008; Hamer et al., 2004) even longer than 10,000 years (Swift, 2001), 10-1000 times longer than other SOM sources (Verheijen et al., 2010) and that it remains intact in deep-sea environment up to 13,900 years (Masiello and Druffel, 1998). The stability of biochar depends by the degree of condensation of the aromatic rings (closely referred to the feedstock) and by the biochar particle size, edaphic and climatic conditions under which biochar is exposed and oxidized (Nguyen et al., 2010; Singh and Cowie, 2010; Zimmerman, 2010, Kuzyakov et al., 2009; Lehmann et al., 2009; Nguyen and Lehmann, 2009) but, in particular, to the charring conditions (temperature and exposure time) at which biochar is produced (Lehmann and Joseph, 2009). Higher pyrolysis temperatures yield less but more stable biochar since the proportion of aryl-C to aliphatic-C increases with increasing in charring temperature (Nguyen et al., 2010; McBeath and Smernik 2009; Baldock and Smernik 2002).

The rate at which biochar may be decomposed varies according to the stability of its oxidizable components. Usually, an initial decomposition of the surface labile components of the biochar particles (e.g. aliphatic-C) may occur, followed by a much slower decomposition of condensed aromatic-C, which dominate the biochar core structure (Waters et al., 2011).

1.5 Biochar structural and chemical composition

1.5.1 Structural composition

Generally, a biochar fragment consists of 2 main structural fractions: i) stacked crystalline graphene sheets and ii) randomly ordered amorphous aromatic structures. During pyrolysis, a considerable mass of the initial biomass is lost under volatile forms and a rigid amorphous C matrix remains. Over 120 °C organic materials lose moisture and begin to undergo some thermal decomposition. Between 200 and 260 °C hemicelluloses are degraded, cellulose starts to degrade between 240 and 350 °C and finally, lignin between 280 and 500 °C (Sjöström, 1993). The proportion of aromatic C in the forming biochar increases as the pyrolysis temperature raise due to the relative increases in the loss of volatile matter which occur in the following order: water, hydrocarbons, tarry vapors, H_2, CO and CO_2), and the conversion of alkyl and O-alkyl C to aryl C (Baldock and Smernik, 2002; Demirbas, 2004).

When pyrolysis temperature reaches 330 ºC, polyaromatic graphene sheets start to grow laterally at the expense of amorphous C phases and, finally, coalesce. Over 600 ºC, carbonization is the dominant process and it is distinguished by the removal of most remaining non-C atoms with consequent increases in relative C content that can represent up to 90% of the final biochar (Antal and Grönli, 2003; Demirbas, 2004). Pyrolysis of wood-based feedstocks yield coarser and more recalcitrant biochars with a C content up to 80% since the rigid ligninolytic nature of the original biomass is retained (Winsley, 2007).

1.5.2 Chemical composition and surface chemistry

Biochar is a highly heterogeneous material and its major constituents are C, ash, volatile matter, minerals and moisture (Tab. 1.2) (Sohi et al., 2009; Antal and Grönli, 2003).

Table 1.2. Range of relative composition for biochars obtained from different feedstock and different pyrolysis conditions.

Compound	**Ranges of relative proportion (w w^{-1})**
C	50-90
Moisture	1-15
Volatile matter	0-40
Ash	0.5-5

Source: Brown (2009) and Antal and Grönli (2003)

The relative proportion of biochar components affects its chemical and physical behavior (Brown, 2009), its suitability for a site specific application, its transport and fate in the environment (Downie et al., 2009). The complex and heterogeneous chemical composition of biochars is extended to its surface chemistry which basically is the most reactive part of each biochar fragment that could explain how biochar interacts with organic and inorganic compounds in the environment. Physical structure and chemical bounds in the biomass are broken and re-assembled during pyrolysis resulting in the formation of numerous functional groups (e.g. hydroxyl -OH, amino-NH_2, ketone -OR, ester -(C=O)OR, nitro - NO_2, aldehyde -(C=O)H, carboxyl -(C=O)OH) mainly positioned on the outer surface of the graphene sheets (Harris, 1997; Harris and Tsang, 1997) and porous surfaces (Van Zwieten et al., 2009).

Depending on the feedstock and charring temperature, some of these functional groups can take up (acceptor) and release (donor) several hundred micromoles of electrons per gram (Klüpfel et al., 2014) resulting on coexisting areas which properties can range from acid to basic and from hydrophilic to hydrophobic (Amonette and Joseph, 2009) properties with important implications in soil cycles. Results from Kappler et al. (2014) suggest that biochar can alter soil biogeochemistry either indirectly by changing the soil structure and chemistry or directly by mediating electron transfer processes (i.e., by functioning as an electron shuttle). Besides, elements such as H, O, N, P and S are predominantly incorporated within the aromatic rings and defined as heteroatoms (Bourke et al., 2007) which are thought to be of great contribution to the highly heterogeneous surface chemistry and reactivity of biochar.

1.5.3 Particle and pore size distribution

Feedstock and charring conditions mainly establish the physical make-up of biochar in terms of particle size and pores distribution (Cetin et al., 2004). However, shrinkage and frictions occurring not only during processing, but also during transport, storage, manipulation and distribution to soils reduce particle sizes of incorporated biochars.

Generally, wood-based feedstocks originate coarser biochars whereas crop residues and/or manures generate finer fragments with a weaker structure (Sohi et al., 2009). The pyrolysis technology (e.g. reactor type and shape), conditions (heating rate, max temperature, residence time, pressure, flow rate), pre- (e.g. drying, chemical activation) and post- (e.g. sieving, activation) treatments greatly affect biochar physical structure (Brown et al., 2009; Cetin et al., 2004; Lua et al., 2004; Antal and Grönli, 2003). Particle and pore size were found to decrease as the pyrolysis temperature increases (Schimmelpfennig and Glaser, 2012; Downie et al., 2009); for instance, higher heating rates and shorter residence times, resulted in finer biochars

(Cetin et al., 2004) while slow pyrolysis (heating rates of 5-30 ºC min^{-1}) can use larger feedstock particles, thereby producing coarser biochars (Downie et al., 2009). The latter are also nutrient-poor and less susceptible to microbial degradation in the environment (Sohi et al., 2009). Biochars produced from crop residues (e.g. rice husk), manures and seaweed are generally finer and less robust than obtained with wood-based feedstocks (Winsley, 2007). Larger biochar particles, as a result of melting followed by fusion, can also be obtained by increasing the flow pressure up to 20 bars during pyrolysis (Cetin et al., 2004).

Biochar has a high porous structure (Fig. 1.4) and, as anticipated, feedstock and charring conditions are the main factors determining its pore size distribution, total surface area (Downie et al., 2009) and bulk density (0.3 Mg m^{-3} as compared to the average of soil bulk density of 1.3 Mg m^{-3}).

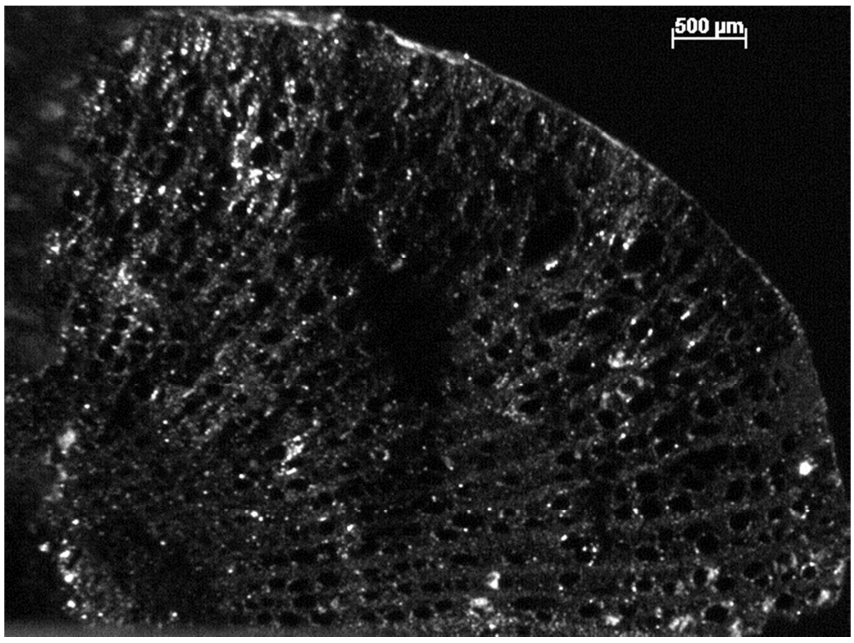

Figure 1.4. Cross section of a highly porous biochar fragment from hardwood. Pores arrangement reproduces the vascular structure of the original biomass.

(Picture: Sorrenti, 2014)

An extensive pore network in biochar is originated during the thermal decomposition of biomasses as a consequence of voids remaining after volatilization of organic compounds at relatively high temperatures. However, the basic porosity and structure of biochar replicate the vascular frame of the original biomass since the cell wall structure (mineral and C skeleton) is retained during the thermal conversion in the formed-biochar (Downie et al., 2009; Laine et al., 1991; Wildman and Derbyshire, 1991).

Downie et al. (2009) classified biochar pores according to their internal diameters (ID) and three categories are typically identified: macropores (ID >50 nm), mesopores (2 nm< ID <50 nm) and micropores (ID <2 nm). The residual cell wall structures of the biomass contribute to the majority of macroporosity in biochar (Wildman and Derbyshire, 1991), while microporosity provides the large surface area which characterizes charred biomasses (Brown, 2009). Particle size distribution and porosity in biochar have implications for both determining the suitability for a specific purpose (Downie et al., 2009) and for the choice of the most adequate application method.

1.6 The Biochar benefits

Over the C-sequestration potential, biochar might yields several co-benefits, some of them listed below with relative references for widening. However, the implications of biochar in agriculture will be discussed in more details in the next chapter:

- ✓ biochar allows to sustainably dispose organic wastes (reduction of social costs and environment advantages) (Takolpuckdee, 2014; Xie et al., 2014; Stavi and Lal, 2013; Ippolito et al., 2012; Xu et al., 2012; Kwapinski et al., 2010);
- ✓ it can be used as a source of renewable bioenergy (Abdullah et al., 2010; Kwapinski et al., 2010; Shackley et al., 2010; Sohi et al., 2009; Laird, 2008; Mathews, 2008; Lehmann, 2007b);
- ✓ it reduces soil GHGs emissions (Zhang et al., 2012; Feng et al., 2012; Kamman et al., 2012; Castaldi et al., 2011; Liu et al., 2011; Van Zwieten et al., 2009);
- ✓ it improves soil physical characteristics and fertility (Mukherjee and Lal, 2013; Ventura et al., 2012; Atkinson et al., 2010; Van Zwieten et al., 2010; Rondon et al., 2007; Liang et al., 2006; Oguntunde et al., 2004; Lehmann et al., 2003; Glaser et al., 2002);
- ✓ it stimulates soil microbial biomass activities and increases biodiversity (Rutigliano et al., 2014; Watzinger et al., 2014; Ameloot et al., 2013; Luo et al., 2013; Anderson et al., 2011; Castaldi et al., 2011; Khodadad et al., 2011; Lehmann et al., 2011; Solaiman et al., 2010; Steinbeiss et al., 2009);
- ✓ it promotes root mycorrhizal colonization (Mau and Utami, 2014; LeCroy et al., 2013; Warnock et al., 2007);
- ✓ it reduces the losses of nutrients as well as chemicals run-off (Ventura et al., 2013; Major et al., 2012; 2009; Yao et al., 2012; Knowles et al., 2011; Laird et al., 2010);
- ✓ It improves nutrient–use efficiency (Ippolito et al., 2012; Van Zwieten et al., 2010);

- ✓ it reduces the bioavailability and phytotoxicity of heavy metals and pesticides in soils (Ogbonnaya and Semple, 2013; Park et al., 2011; Kookana, 2010; Mohan et al., 2012; 2007; Wang et al., 2008);
- ✓ it improves soil water-holding capacity (Baronti et al., 2014; Yu et al., 2013; Sohi et al., 2010);
- ✓ it can increase plant growth and crop yields (Kamman et al., 2013; Spokas et al., 2012; Jeffery et al., 2011; Sohi et al., 2010; Major et al., 2010; Steiner et al., 2007).

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CHAPTER 2

BIOCHAR IN AGRICULTURE

2.1 Biochar mechanisms in soil

Biochar addition has been indicated as a strategy able to influence several soil physical, chemical and biological properties (Ippolito et al., 2012) due to its intrinsic properties that develop over time through surface oxidation and its interaction with plant–soil–microbial components (Spokas et al., 2012; Downie et al., 2009).

Biochar added to soil acts as a sponge, soaking up different forms of organic matter (OM) as well as water and nutrients (Glaser et al., 2002). However, it is still unclear if adsorbed compounds may serve as nutrients, if the porous biochar increases nutrient immobilization, if it provides niches for plant symbionts such as arbuscular mycorrhiza fungi (MF), which may provide nutrients to plants or a combination of all mechanisms occur (Scheifele et al., 2014). Literature reports a number of benefits associated with biochar addition to cropping soils, in particular where fertility is depleted and productivity declines. The addition of biochar to soils increases the size of stable soil carbon (C) pools and may potentially induce multiple functions: source of OM, increase of plant-available nutrient, increase of soil water holding capacity (WHC), alter physical characteristics (i.e. bulk density), enhance cation exchange capacity (CEC), stimulate microbial activity and biodiversity and reduce GHG's emissions (Spokas et al., 2012; 2010; Kookana et al., 2011; Jeffery et al., 2011; Atkinson et al., 2010; Sohi et al., 2010; 2009; Verheijen et al., 2010). These properties may act simultaneously to create a positive feedback loop in soil fertility; more biochar means more water and nutrients in soil, which increase the OM input into the soil that can be absorbed onto the biochar surface or stored in stable soil C reservoirs (Lehmann et al., 2007).

To explain how biochar in soil might benefit plant growth and crop yield, generally four mechanisms are being proposed (Lehmann and Joseph 2009; Sohi et al., 2010; 2009):

- ✓ alteration of soil chemistry (direct source of nutrients or improvement of nutrient efficiency);
- ✓ mitigation and/or removal of soil constraints (e.g. low pH, aluminum (Al) toxicity, contaminants) which may limit plant growth;
- ✓ modification of the nutrient dynamics in soil and/or altering soil reactions by providing chemically active surfaces;

- ✓ altering soil physical parameters that benefit root growth and/or nutrient, water retention and uptake;

The first mechanism can occur when biochar provides a significant and plant-available mineral content (in particular as ash) or increases the soil CEC, while the second mechanism can explain, at least in part, benefits observed in acid or contaminated soils either by heavy metals and/or organic pollutants.

The last two mechanisms depend on the biochar physical persistence (and changes) and stability in soil and include the impact on soil WHC, surface area, bulk density, nutrient leaching etc.

Evidently, more than one mentioned mechanisms can occur at the same time. Rate and extent of each mechanism are hardly quantifiable and depend on many factors (i.e., feedstock characteristics, charring conditions, application rate, initial soil texture and fertility, water availability, genetic resources) and, generally, evolve over time as the chemical and physical process affecting biochar fragments in soil, result in a gradually increase of the concentration of smaller and partially-oxidized particles with implications for functional interactions in the soil environment (Cheng et al., 2008; 2006).

Furthermore, biochar may have the potential to sustain similar crop yield while reducing nutrient application rate, with environmental benefits (i.e. reduction of nutrient leaching and GHGs emission). The purpose of biochar application might be not only to increase yield, but possibly to achieve predictability in yield through a lower susceptibility to climatic events such as floods and drought (Sohi et al., 2009). Also, biochar could increase, maintain or at least limit gradual decrease in crop yield on lands where soil fertility and productivity is currently in decline (Sohi et al., 2009).

2.2 Impact of biochar on soil properties and implication on plant growth

2.2.1 Key functions of biochar in soil

2.2.1.1 Soil structure

When incorporated into the soil, biochar can alter soil physical properties (i.e. texture, pore size distribution) with possible implications on soil tensile strength (compaction), aeration, permeability, infiltration and soil hydrology (i.e. WHC), thermal properties, microbial activity and root growth (Bruun et al., 2014; Chen et al., 2011; Laird et al., 2010a, Atkinson, et al., 2010; Downie et al., 2009; Oguntunde et al., 2008). These effects may be temporary or last longer. Generally biochar reduces the soil bulk density (Laird et al., 2010a) and if incorporation of large biochar fragments (e.g. > 0.5 mm) occurs, it increases soil aeration and

reduces anoxic microsites, with implications on SOM mineralization rates, nitrification-denitrification dynamics and GHG's emissions (Cayuela et al., 2013; Ball et al., 2010; Sohi et al., 2010). From an agronomical point of view, application rates of even 1-2% (w w^{-1}) of biochar may significantly decrease bulk density in soil, which is considered beneficial for plant growth since it is associated with higher SOM content then to a higher soil WHC and to a lower soil compaction (Mukherjee and Lal, 2013; Chan et al., 2007).

2.2.1.2 Soil pore size, distribution and surface area

When biochar is applied to the soil, it may contribute to alter the physical nature of the system. Biochar may significantly influence soil texture, structure, porosity and consistency through changing the bulk surface area, pore size distribution, density and packing (Downie et al., 2009), thereby biochar may influence soil properties such as aggregation (and workability), response to water, shrink/swell dynamics and permeability (Brady and Weil, 2008). After biochar incorporation, soil porosity usually increases, although an alteration of soil hydrology (decline of water infiltration rates) has been observed as a response of partial or total blockage of soil pores by the smallest particle size fraction of biochar. In fact, biochar has generally a poor mechanical strength, hence it may collapse into smaller particles as a consequence of both biotic (e.g. microbial degradation) and abiotic (e.g. climate, tillage) stresses. Smaller fragments may fill up small soil pore spaces, thus increase in soil bulk density (soil compaction) may occur over time.

Physical soil changes induced by biochar addition may have both direct and indirect implications on plant growth since a lower soil compaction facilitate a deeper root penetration and a wider radial expansion, while air and water availability within the root zone is promoted (Bruun et al., 2014).

Evidences suggest that biochar addition increases net soil surface area (SA) (Chan et al., 2008) up to 4.8 times (Liang et al., 2006). Laird et al. (2010a) reported an increase in specific SA from 130 to 150 m^2 g^{-1} when biochar derived from mixed hardwoods was applied to a clayey soil at a rate of 20 g kg^{-1}. The increase in SA has positive implications on soil WHC, soil aeration and may benefit native microbial communities as well as sorption potential (Van Zwieten et al., 2010a; Verheijen et al., 2010). In fact, SA is associated with the formation of bonds and complexes with cations and anions, metals and other elements in solution affecting the nutrient retention capacity (Atkinson et al., 2010; Hammes et al., 2009; Liang et al., 2006).

2.2.1.3 Soil color, albedo and changes in soil temperature

Solar radiation (as affected by the impacting incidence angle and vegetation cover), specific soil heating rate and water content, mainly control the dynamic of soil warm up in spring, whit implications on the emergence and growth of seedlings. The main factors controlling specific heat are soil color and moisture content. The specific heat of pure water and dry soil is about 4.18 and 0.8 J g^{-1} K^{-1}, respectively. Anthrosols profile of Terra Preta de Indio sites shows that high concentration of charcoal darken soil color (Fig. 1.2), thus anthropic biochar application has been suggested to alter soil albedo which is the diffuse reflectivity or reflecting power of the soil surface measured as the ratio between the reflected radiation from the surface and the incident radiation upon it. Land surface albedo represents an important component of global and regional climate models since it influences climate and drives weather. For instance, soil albedo changes due to natural (i.e. snowfall) or anthropogenic activity (i.e. charcoal or compost addition) may have a local temperature feedback, thus potentially altering the climate at microscale. As snowfall increases local albedo (reflecting sunlight) leading to local cooling, a darken soil may led to a local warming.

The degree of soil darkening due to anthropic biochar addition depends on: 1) native soil color before biochar addition, 2) biochar color and application rate, 3) method and depth of soil incorporation, 4) soil surface roughness and, 5) changes in water holding retention at the soil surface site (Verheijen et al., 2010). The darken soil color in charcoal sites decreased the Munsell value (in colorimetry, the Munsell color system is a color space that specifies colors based on three color dimensions: hue, value (lightness), and chroma (color purity. Value indicates the lightness of a color. The scale of value ranges from 0 for pure black to 10 for pure white) compared with adjacent soil from 3.1 (± 0.6) to 2.5 (± 0.4) (Oguntunde et al., 2008) and for this reason the soil surface temperature likely increased in a biochar-amended soil while no differences were detected at 0.075 m depth in an apple orchard (Ventura et al., 2013). However, the effect of biochar on soil albedo changes and related warming implications should be more pronounced in bare soils well exposed to solar radiation than in orchards, since in the former case, the solar radiation reaching the surface is low in winter and it is reduced by canopies and cover crops during the vegetative season.

Although biochar-amended soils are usually darken in color, the higher water content in biochar treated soils could offset the extra energy absorption, resulting in a more slowly soil warm up (Brady, 1990) compared to unamended soils. This implies that biochar with water repellent properties (e.g. low water retention capacity) might induce the greatest increase in soil warming potential with a greater impact in light-coloured soils.

2.2.1.4 Soil cation exchange capacity (CEC) and pH

Soil CEC characterizes the ability of a soil to retain nutrients and ions (i.e. NH_4^+, Ca^{2+}, Mg^{2+}) between charged particles through electrostatic forces. Retained nutrients are then potentially exchanged with the soil solution. Soils amended with biochar show an increased CEC (Liang et al., 2006). High soil CEC prevents nutrient losses by leaching (reducing groundwater contamination), and improves nutrient availability for plant uptake, potentially increasing nutrient use efficiency (Downie et al., 2009).

The increase in CEC in biochar amended soils seems to be related with a higher surface charge density (Van Zwieten, et al., 2010a) and to a slow biological oxidation of the aromatic C compounds with formation of carboxyl groups on the edges of the condensed aromatic skeleton that occurs on the biochar surfaces when applied to soils (Liang et al., 2006; Glaser et al., 2002). However, feedstock and pyrolysis conditions greatly affect biochar surface charge properties (Singh et al., 2010a). Generally, cations in soil might be bounded by ion and covalent bindings to negatively charged sites located on clay and OM particles (as well as on the reactive surface of biochar). On the other hands, anions (i.e. NO_3^- and phosphates) are weakly bounded to the positively surface charge of clay. The adsorption of highly oxidized OM particles may induce the development of negative charges onto the biochar surface. As a result, the original positive exchange sites on biochar surface may decline and negative charge sites develop with biochar ages (Cheng et al., 2008). Moreover, fresh biochar is typically hydrophobic and contains polar functional groups at the surface which evolve in more carboxylic and phenolic groups after exposure to the environment (water and oxygen in the soil) (Cheng et al., 2008), becoming more hydrophilic with time. As a result, both above mentioned changes contribute to explain the long-term positive effect of biochar in holding cations (Cheng et al., 2008; 2006).

The pH of biochar usually ranges from slightly acidic to alkaline (Chan and Xu, 2009) and this is mainly due to the relative high abundance of minerals with alkaline properties such as phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and manganese (Mn) which require higher temperatures to volatilize (above 700 ° C) compared to the levels at which most biochar are produced (<600°C).

The addition of biochar may increase soil pH resulting in a liming effect providing, therefore, some benefits in neutralizing acidic soils. However, this ability depends on both the feedstock and charring conditions since, for instance, increasing pyrolysis temperatures generally led to biochars with higher pH (Singh et al., 2010a). In acid soils, changes in soil pH influence the bioavailability of toxic elements. Van Zwieten et al. (2010a) measured a reduction in

exchangeable Al from 2 to <0.1 cmolc kg^{-1} in concomitance of an increased soil pH upon two different papermill waste biochars application. Likewise, Kloos et al. (2014) observed a pH increase in three different soils using biochars made either of wheat straw, mixed woodchip or vineyard pruning. Soil-applied biochar increased soil pH by 0.36 and 0.75 units with and without fertilizer, respectively, in acid soil (Lehmann et al., 2003).

Increase the pH in acid soil may also stimulate microbial activity and, with this, a priming effect (decomposition of pre-existing OM) may occur as a consequence of biochar application. The modification of soil pH may be, at list in part, the explanation of most of the benefits observed on plant growth and productivity after biochar addition in weathered soils (Jeffery et al., 2011), which in turn can indirectly induce also an increase of the amount of C added to the soil through residues and root exudates.

2.2.1.5 Soil water holding capacity (WHC)

Altering soil hydrology, biochar has the potential to provide a long-term modification in water cycling and ecosystem processes mediated by water, thereby changes on soil WHC (measured as the amount of water retained by a soil that has been saturated and then allowed to freely drain for a specific amount of time) are expected. Direct consequence of biochar amendment on soil hydrology may include changes in infiltration and drainage rates, shifts in the amount of water stored in soils, including water stored in a plant-available form, and shifts in soil hydrophobicity. Overall benefits induced by biochar on the soil WHC seem to be mainly attributed to its porous structure (Fig. 2.1), which reflects the cellular arrangement of the original feedstock (Sohi et al., 2010). Studies on soil-biochar mixtures have shown an increase in soil water-holding capacity up to 30% (Basso et al., 2013; Kinney et al., 2012; Novak et al., 2012; Lei and Zhang, 2013). Some authors ascribed the improved moisture retention as the key factor for the positive plant response (Kookana et al., 2011; Atkinson et al., 2010; Sohi et al., 2010) and for the improved plant water use efficiency (Baronti et al., 2014; Downie et al., 2009).

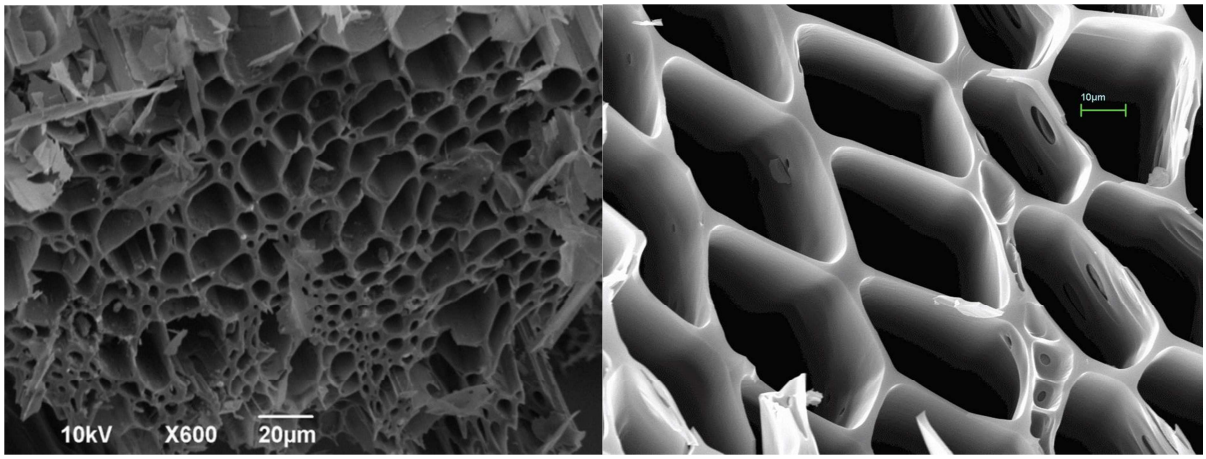

Figure 2.1. Scanning electron microscopy (SEM) of biochar fragments from Miscanthus (left) and pine (right). Differences in pore size are evident, according to the vascular arrangement of the original feedstock. (source: courtesy of Carbolea Research Group, www.carbolea.ul.ie (left) and Feltz, 2010 (right).

Glaser et al. (2001) report that WHC of Terra Preta de Indio soils was 18% higher than in adjacent soils in which charcoal was absent and attributed this to the increased surface area and porous structure of the char particles. Biochar generally increases soil WHC with as low as 0.5% (g g^{-1}) biochar application rate sufficient to improve the ability of plants to survive under drought conditions (Bruun et al., 2014; Kinney et al., 2012; Kammann et al., 2011; Uzoma et al., 2011; Brockhoff et al., 2010; Asai et al., 2009).

These effects are confirmed by many studies. WHC doubled on a loamy sand soil using a woody biochar amendment (yellow pine from pyrolysis at 400°C) at a rate of 9% (Yu et al., 2013). In a column study, Laird et al. (2010a) measured up to 15% in increasing water content in a biochar-amended Clarion soil. Biochar from black locust (*Robinia pseudoacacia*) increased the WHC by 97% and saturated water content by 56%, but reduced hydraulic conductivity (Uzoma et al., 2011). However, some experiences have also reported no significant changes in WHC for some biochar-soil combinations (Laird et al., 2010a; Kinney et al., 2012; Abel et al., 2013). For instance, a decreasing in moisture content in a clayish soil was reported after biochar addition by Verheijen et al. (2010). Possible mechanisms to explain the latter response could be that biochar replaced clay particles with an higher water retention capacity (Verheijen et al., 2010) or that hydrophobic biochar caused preferential leaching flows or decreased water infiltration (Major et al., 2010a).

However, the effect of the biochar addition on the WHC is not always predictable. The moisture release curve (referred as the usual measure to characterize soil pore size distribution

by showing the kinetic of the soil moisture under increasing tensions) in a biochar-amended loamy-sandy soil was unaffected when biochar was added up to 22 t ha^{-1}, while only at the highest rate (88 t ha^{-1}) at water potentials in the range of 0.01–0.20 MPa a significant effect occurred. In details, only at the highest water potential, the volumetric water content in biochar-added soil was double compared to unamended soil (Gaskin et al., 2007).

The beneficial influence of biochar on soil WHC has been mainly linked to its porous structure which can absorb and retain water (Verheijen et al., 2010; Downie et al., 2009). Nevertheless, a further explanation could be due to biochar induced changes in the distribution and connectivity of pores in the soil environment. The impact of biochar on soil texture at the macroscale (macroporosity) is often short-lived as biochar is physically divided rapidly in soil into smaller particles, similar to silt size (Brodowski et al., 2007), presumably by abrasion or by the shrink-swell, freeze-thaw cycles, etc. (Sohi et al., 2009). Thus, considering that pore size of biochar is relatively fixed and that porosity of mineral soil is mainly controlled by texture, it may be a longer-term positive effect of biochar-addition on available moisture in sandy soils typically dominated by much larger pores than in biochar. The effect is expected to be neutral in medium-textured soils and potentially detrimental to moisture retention in clay soils (Tryon, 1948). This behavior seems related to the hydrophobic nature of biochar and, in particular, to the alteration of the soil pore size distribution (Glaser et al., 2002; Tryon, 1948).

2.2.1.6 Microbial biomass

Mixing biochar with soil often induces stimulation of the microbial biomass, alters the community biodiversity and activates dormant soil microorganisms (Hu et al., 2014; Gomez et al., 2013; Anderson et al., 2011; Khodadad et al., 2011; Lehmann et al., 2011; Grossman et al., 2010; Hilscher et al., 2009; Kuzyakov et al., 2009; Steiner et al., 2008a; Knicker, 2007; Hamer et al., 2004) resulting frequently in significant increases in microbial respiration rates (Smith et al., 2010; Hilscher et al., 2009; Steinbeiss et al., 2009; Hamer et al., 2004). Recent findings reveal a pronounced impact of biochar in the short time period on soil microbial community composition and an enrichment of key bacterial and fungal taxa, such as *Actinobacteria*, *Trichoderma* and *Paecilomyces* (Hu et al., 2014). However, a significant inhibition of the soil microbial biomass, along with a slower N mineralization rate, was reported for a coarse textured soil after addition of Eucalyptus biochar-derived (Dempster et al., 2012), suggesting that interaction biochar-microbes could be site and feedstock specific.

The improvement of the habitat for microorganisms in soils may also be a consequence of indirect benefits induced by biochar on physical and chemical soil properties such as increased

soil organic C, increased pH, CEC and WHC, reduced content of exchangeable Al and Mn and sorption of toxic compounds harmful for microorganisms (Jeffery et al., 2011; Karhu et al., 2011; Graber et al., 2011a, 2011b; Verheijen et al., 2010; Van Zwieten et al., 2010b; Loganathan et al., 2009; Qiu et al., 2009; Chan et al., 2008; Yamato et al., 2006; Glaser et al., 2002). After biochar addition, increased CO_2 fluxes from soil may outcome from (i) biotic consumption of C fractions released by biochar (Bruun et al., 2011; Cross and Sohi, 2011; Zimmerman et al., 2011), (ii) abiotic release of biochar-C (Bruun et al., 2008; Cheng et al., 2006;) and/or (iii) interactions between biochar and native SOM pools (priming) (Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011; Kuzyakov et al., 2009).

Labile organic C fractions and soluble nutrients supplied by biochar may be beneficial for the microbial communities (Ameloot et al., 2013) and upon addition of biochar to soil mineralization may be stimulated. This could contribute to explain N retention in soils amended with biochar as a consequence of microbial N immobilization and increased nitrates recycling due to higher availability of C. However, Thies and Rillig, (2009) suggested that the interactions between microbes and biochar include also the attraction of microbes by the molecules absorbed on the biochar surface, such as OM fractions, minerals and nutrients as well as extracellular enzymes. Pietikäinen et al. (2000) reported that biochar adsorbed up to 42% of dissolved organic C (DOC) from a litter extract, which consequently attracted a large community of micro-organisms. The reduced bioavailability of various soil toxins through the adsorption of phytotoxic phenolic compounds by charcoal (Graber et al., 2011a,b) has also been suggested as one of possible mechanisms for a promoted nitrifying activity by micro-organisms in forest soils (MacKenzie and DeLuca 2006; Berglund et al., 2004; Zackrisson et al., 1996), for a reduction of organic compounds that could trigger N immobilization (DeLuca et al., 2006) and for an indirect stimulation of soil microorganism colonies (Ameloot et al., 2013).

Biochar may provide favorable microsites and secure environments for microbial colonies to prosper (Fig. 2.2), including bacteria (Pietikäinen et al., 2000) and mycorrhizal fungi (MF) (Ezawa et al., 2002; Saito and Marumoto, 2002) as well as shelter against predatory soil fauna and desiccation (Lehmann et al., 2011; Steinbeiss et al., 2009; Warnock et al., 2007).

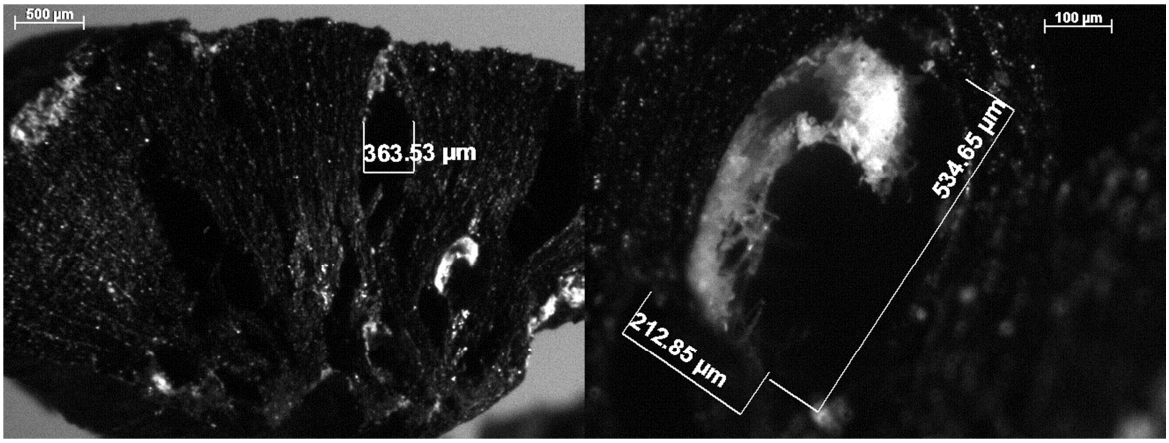

Figure 2.2. Microscopic images of a cross section biochar fragment from hardwood recovered from the field after 4 years of environmental exposure. Pores may represent potential shelters for a large variety of edaphic microorganisms, as suggested by an unknown colony of microorganisms (right) retrieved in the pores. *(Pictures: Sorrenti, 2014).*

Nevertheless, the average pore size of biochar (nm scale) is often much smaller than that of the smallest soil organisms (µm scale) (Hassink et al., 1993) and this could explain why Quilliam et al. (2013) did not observe a significant colonization of the biochar pores by soil microbes after its incorporation, at least until the labile fractions where depleted and larger pores were provided (after 3 years).

Biochar has also been indicated as a promoting agent of the symbiotic associations between arbuscular MF and terrestrial plants (Mau et al., 2014; Vanek and Lehmann, 2014; O'Neill et al., 2009; Thies and Rillig, 2009; Steiner et al., 2008a; Warnock et al., 2007; Pietikäinen et al., 2000; Zackrisson et al., 1996) and such interaction might be responsible for increase of soil nutrient availability and enhance disease tolerance (Downie et al., 2009), although it has been proposed that biochar could potentially supply substances that might inhibit microbial activity (De Luca et al., 2006). However, only few studies report negative effects on MF with biochar addition (Warnock et al., 2007; Gaur and Adholeya, 2000). Arbuscular MF in soil may have a great impact on plant nutrition through improved P and Mg availability via extensive fungal hyphae system and by the mineralization of organic N into mineral forms, available either to plants or susceptible to volatilization (Major et al., 2009). Makoto et al. (2010) indicated that an increased plant P uptake, due to the utilization of phosphate released by the MF and seedling root/biochar contact, was the responsible for an increase of both root growth and aboveground biomass of *Larix gmelinii* (*Gmelin larch*) when biochar was applied along with

MF, while an increase in maize root mass and colonization rates of MF was observed by Yamato et al. (2006), using acacia bark charcoal in Indonesia.

Similarly, Rondon et al. (2007) indicated that biological N_2 fixation (BNF) in soil seems to be promoted after biochar addition, as observed on *Phaseolus vulgaris* which increased the BNF by 49 and 78% with 3 and 6% (w w^{-1}) biochar rates, respectively. However, the same study concluded that a greater B and Mo availability supplied with biochar could explain, at least in part, the increased BNF.

In forest soils, increased N mineralization and nitrification rates were attributed to biological processes stimulated by charcoal amendment (Ball et al., 2010; MacKenzie et al., 2008; Berglund et al., 2004). On the contrary, in cultivated soils, N availability may be reduced after biochar addition due to either N immobilization as a consequence of N-poor biochar with high C/N ratio and the adsorption of available NH_4^+ on the char surface (Lehmann et al., 2006). Kolb et al. (2009) described an enhanced microbial biomass and activity and a decreased extractable N along with increasing biochar rates in three agricultural soils using a manure-pine biochar. Nevertheless, N immobilization in soil is not always a consequence of the biochar application, since C in charred biomass is highly recalcitrant thus it is not expected to immediately enter the C cycle (Major et al., 2009).

2.2.1.7 Impact of biochar on greenhouse gas emission

CO_2, methane (CH_4) and nitrous oxide (N_2O) are the main contributors to GHGs in the atmosphere (Lal, 2008). The two latter gases are estimated to be 310 and 21 times (over a time horizon of 100 years) stronger GHGs than CO_2, respectively (Forster et al., 2007), although CO_2 is still by far the most significant GHG (Verheijen et al., 2010). CH_4 and N_2O are both produced by the soil microbiota communities, but while the first is produced under anaerobic conditions through the methanogenesis process, N_2O is a partial product of the N cycle (nitrification and denitrification processes) (Cayuela et al., 2013; Van Zwieten et al., 2009). Various anthropogenic activities, such as fossil fuel combustion and industrial processes as well as agronomic practices (drainage of wetlands, plowing, land use conversion, rice (*Oryza sativa*) paddy fields, use of fertilizers, livestock and wetlands) represent important sources of GHGs (Yao et al., 2012a). Eighty percent of emitted N_2O and 50% of CH_4 are originated by soil processes in managed ecosystems (Gaunt and Cowie, 2009).

In soils amended with biochars, no impact or even an increase in the emissions of GHGs fluxes under field trials or laboratory incubation studies were observed (Jones et al., 2011; Scheer et al., 2011; Wardle et al., 2008). For example, after biochar addition, a short-term increase in the

soil CO_2 flux has been measured (Bruun et al., 2008; Steiner et al., 2008; Hamer et al., 2004) and the effect was related to both biotic (microbial stimulation as a consequence of the labile C-fractions supplied with biochar) and abiotic processes (i.e. increased WHC) (Smith et al., 2010). Up to 23% of the biochar-C content was quickly mineralized, leading to an increase of CO_2 emission from soil in a column incubation study (Rogovska et al., 2011). Nonetheless, such effects did not last more than few months, after which biochar amended soils stabilized their CO_2 flux to similar rates of those unamended. Besides, an increasing in CO_2 emission from biochar amended soils could be a consequence of the improved soil WHC which promotes decomposition of native SOM (Wardle et al., 2008). However, according to Jones et al. (2011), the initial short-term increasing CO_2 emission from soil, with consequent fast C loss, is comparatively negligible compared to the amount of C stored with the biochar, thus should not affect the C sequestration potential of biochar on a long-term basis.

In summary, CO_2 flux from biochar amended soil can be initially stimulated because of: a) microbial decomposition of labile soluble C-compounds present on biochar (Smith et al., 2010); b) microbial respiration of abiotically released inorganic C (Jones et al., 2011; Zimmermann et al., 2010) and c) a "priming effect" which is an extra decomposition rate of native OM following biochar application (Wardle et al., 2008).

Mechanisms responsible of N_2O emission from soil, including nitrification and denitrification (Baggs, 2008), can be altered by biochar (Singh et al., 2010b; Van Zwieten et al., 2010b; 2009). Emissions of N_2O from biochar-amended soils have been shown to decrease both from field and incubation studies (Case et al., 2012, Yao et al., 2012a, Zhang et al., 2012a, 2012b ; Rogosvka et al., 2011) although there are examples where biochar-amended soils stimulated N_2O emissions, as observed by Singh et al. (2010b) who measured an initial N_2O enhancement due to the higher labile N content of biochar which promoted the microbial activity. This effect, however, decreased over time.

Mechanisms responsible for N_2O reduction following biochar application are attributed to the influence of biochar on soil hydrology (increased soil aeration) (Van Zwieten et al., 2010b; 2009; Yanai et al., 2007), to the presence of microbial inhibitor compounds such as ethylene (Spokas et al., 2010) and to changes in nitrification-denitrification processes (physical or biological immobilization of NH_4^+ or NO_3^-) (Singh et al., 2010b; Van Zwieten et al., 2010b). Yanai et al. (2007) reported that biochar-amended soils (100 g kg^{-1} dw soil) reduced N_2O emissions by 89% as a consequence of an increased soil aeration, while Van Zwieten et al. (2010b) showed reduced N_2O emissions using different biochars in an incubation study and suggested that such effect was likely due to an increased adsorption of nitrate ($N\text{-}NO_3$) by the

biochar surfaces. Expressed as CO_2 equivalents, biochar addition significantly reduced GHGs emissions only in the N-fertilized silt-loamy soil by decreasing N_2O flux (Zheng et al., 2012). However, relatively high biochar rates are required before N_2O emission is limited, as suggested by Spokas et al. (2009) who reported a reduction in the N_2O emissions by up to 74% only when large biochar rates (20, 40 and 60%) were applied to the soil, whereas no suppression was observed at smaller rates.

As a result of biochar addition, a change in the emissions of CH_4 from soil has also been observed (Spokas et al., 2009; Rondon et al., 2005). In a chinese rice paddy amended with a wheat straw biochar-derived at a rate of 10 and 40 Mg ha^{-}1, CH_4 emission increased by 31 and 49%, respectively, while in the same trial N_2O emission decreased by 50 and 70%, respectively (Zhang et al., 2012a). On the other hand, CH_4 and CO_2 emissions were reduced by 51 and 91% respectively, in a paddy soil amended with biochar obtained from bamboo and rice straw (Liu et al., 2011). Similarly, CH_4 emission was reduced in a tropical acid soil treated with biochar from mango trees (Rondon et al., 2006). Likewise, an incubation study demonstrated reduction in the emission of all the three main GHGs from three different soil types amended with most of 16 types of biochars (Spokas and Reicosky, 2009).

Different mechanisms have been suggested to be responsible for the reduction of CH_4 emission from biochar amended soil: a) the increase in soil aeration and changes in soil hydrology as anoxic conditions may increase oxidation of CH_4 (Van Zwieten et al., 2010b) b) the higher rate of CH_4 diffusion and oxidation by methanotrophs microorganism activity in well drained soils (Dalal et al., 2008) and c) a stimulatory biochar effects on methanotrophs which assimilate methane-C, subsequently utilized by other organisms via microbial food chain (Feng et al., 2012).

Nevertheless, explanations behind changes in GHGs fluxes following biochar application are often contradictory and seem to be soil/biochar specific. Furthermore, the extent at which biochar affects soil CO_2 emission, obviously depends on biomass type, pyrolysis conditions (temperature and residence time), soil characteristics, climatic conditions and microbial community (Van Zwieten et al., 2009), but specific mechanisms governing such effects remain still unclear (Case et al., 2012; Castaldi et al., 2011) and apparently linked to properties of both biochar and soil conditions before application (Van Zwieten et al., 2010b; Yanai et al., 2007).

2.2.2 Impact of biochar on soil mineral content, functions and cycles

2.2.2.1 Biochar as a source of nutrients to plants

Biochar may represent a potential source of macro- and micronutrients beneficial for plants and soil microbial community (Downie et al., 2009), although nutrient release is often negligible (except for some ash-rich biochars; Abdullah et al., 2010) and limited to the first-second year following application. However, to measure the direct nutrient value of biochars, the plant-available fraction is much more important than the total mineral content which is of poor interest. For instance, mineral N ($N\text{-}NO_3$ and ammonium-N ($N\text{-}NH_4$)) in biochar are found in low concentrations, although the total N content may be relatively high (Chan and Xu, 2009). Biochar produced from sewage sludge was found to contain negligible $N\text{-}NO_3$ and $N\text{-}NH_4$, despite of a high total N content of 6.4% (Bridle and Pritchard, 2004). Similarly, mineral N was found to be less than 2 mg kg^{-1} for a poultry manure and green waste chars with a total N of and 20 g kg^{-1} and 1.7 g kg^{-1}, respectively (Chan et al., 2008). Conversely, available K in biochar is often high and available for plant uptake, as frequently reported (Lehmann et al., 2003; Chan et al., 2007).

Except for N, most of the nutrients in the feedstocks, potentially toxic, are largely conserved during pyrolysis and the characteristic temperature at which each element volatilizes is crucial to define the C/N ratio and the nutrient content of the final biochar. Wood-based biochars show high C/N and C/P ratios while in biochars obtained from manures, crops and food wastes these ratios are lower (Kookana et al., 2011). Certain organic C compounds change their structure and start to volatilize at 100 °C (Krull et al., 2009), while most of the N and sulphur (S) compounds volatilize above 200 and 375 °C, respectively, whereby biochar obtained from pyrolysis above these temperatures tend to be relatively depleted in N and S. However, when N-rich feedstocks are pyrolized at relatively low temperature (< 500 °C) biochars may retain up to 50% of the original N content (Bridle and Pritchard, 2004).

Also, biochar ash content increases as retained C from feedstock decreases. Solubilization of ash in soil may result in readily-available nutrients to plants, which can also promote mineralization of native OM, especially in poor fertile soils. Woody feedstocks generally contain low proportions of ash (< 1% weight) whereas biomasses with high mineral content generally yield ash-rich biochar (Demirbas, 2004). For instance, rice husk and rice hulls may contain up to 24% or even 41% by weight of ashes (Amonette and Joseph 2009; Antal and Grønly, 2003), respectively.

The proportion of aromatic C in biochar increases with pyrolysis temperature, while N content peaks at around 300 °C (Baldock and Smernik, 2002). Low pyrolysis temperatures (<500 °C)

promote the relative accumulation of larger proportion of available K, chlorine (Cl) (Yu et al., 2005), silicon (Si), Mg, P and S (Chan and Xu, 2009, Bourke et al., 2007; Schnitzer et al., 2007) because during thermal degradation of the biomass, K, Cl and N vaporize at temperatures <700 °C, while Ca, Mg, P and S vaporize at considerably higher temperatures (Amonette and Joseph 2009) (>1000°C), becoming concentrated as the progressive elimination of the more volatiles C, O and H occurs (Singh et al., 2010; DeLuca et al., 2009; Gaskin et al., 2008). Other relevant minerals can be present in the biomass, such as Si, which is part of the cell walls, mostly in the form of silica (SiO_2) (Verheijen et al., 2010). To this respect, Houben et al. (2013) concluded that biochar could be used as a potential source of bio-available Si for Si-accumulator crops (for instance, in highly weathered tropical soils with low content in C, nutrients and bio-available Si).

Biochars greatly differ each other in term of nutrient content, mineral form and chemical structure according to the pyrolysis conditions and biomass (Tab. 2.1) (Kookana et al., 2011; Singh et al., 2010; Gaskin et al., 2008). During pyrolysis, high temperatures (>800 °C) result in biochars with higher pH, electrical conductivity (EC) and extractable $N\text{-}NO_3$, while low-temperature biochars (<350 °C) show greater amount of extractable P, NH_4 and phenols (DeLuca et al., 2009).

Feedstocks with high nutrient content, such as manures, yield biochars with greater nutrient value compared to vegetal feedstocks (Singh et al., 2010). Biochars obtained from similar feedstocks and pyrolized under temperatures of 400 or 500 °C revealed a higher N and a lower P content (34.7 and 30.1 mg kg^{-1}, respectively) for the lower compared with the higher temperature char (30.9 and 35.9 mg kg^{-1}), respectively (Gaskin et al., 2008).

Physical characteristics (porosity and surface area) may control the release of soluble nutrients from charred biomass and pore connectivity makes progressive nutrient release rather than instantaneous as in the case of ash, which quickly release its mineral content. This behavior could be also associated with mineralization of condensed tars and oils that may occlude biochar pores (Fernandes et al., 2003).

Table 2.1. pH and elemental composition of biochars as affected by feedstock and pyrolysis conditions *(n.d.= not declared)*

Feedstock	pH	C	N	P	K	C/N	Pyrolysis conditions	Source
				g kg^{-1}				
Wood	n.d.	708	10.9	6.8	0.9	65	empiric	Lehmann et al., 2003
Green wastes	9.4	360	1.8	0.2	21	200	450° C	Chan et al., 2007a
Poultry litter	9.9	380	20	25.2	22.1	19	450° C	Chan et al., 2008
Sewage sludge	n.d.	470	64	56		7	450° C	Bridle and Pritchard 2004
Broiler litter	n.d.	258	7.5	48	30	34	700° C	Lima and Marshall, 2005
Bark of *A. mangium*	7.4	398	10.4	n.d.	n.d.	38	260°-360° C	Yamato et al., 2006
Rice straw	n.d.	490	13.2	n.d.	n.d.	37	500° C	Tsai et al., 2006a
Sugar cane bagasse	n.d.	710	17.7	n.d.	n.d.	40	500° C	Tsai et al., 2006b
Coconut shell	n.d.	690	9.4	n.d.	n.d.	73	500° C	Tsai et al., 2006b
Oil malle tree residues	8.4	340	12	7.0	7.0	28	Moki method	Blackwell et al., 2007
Soybean cake	n.d.	590	78.2	n.d.	n.d.	7.5	550° C	Uzun et al., 2006
Eucaliptus deglupa	7.0	824	5.73	n.d.	n.d.	144	350° C	Rondon et al., 2007
Pruned hardwood	9.8	578	9.1	23.3	14.0	63.5	500 ° C	Ventura et al., 2013

Macronutrients

Nitrogen

Vegetal biomasses account for a range of N molecules (compounds), including amino acids, amines, and amino sugars. When pyrolized, these compounds condense, forming heterocyclic N aromatic structures (Cao and Harris, 2010; Koutcheiko et al., 2006). N functional groups detected in a low temperature biochar top surface were mainly pyrrolic or pyridinic amines as measured by X-ray photoelectron spectroscopy (XPS) (Amonette and Joseph, 2009). Nuclear magnetic resonance (NMR) spectroscopy has shown that aromatic and heterocyclic N-containing structures in biochar occur as a result of biomass heating, converting labile structures into more recalcitrant forms (Almendros et al., 2003). N left in biochar obtained by charring vegetal biomasses is often found in low amount (Chan et al., 2007a) and is largely transformed into recalcitrant heterocyclic N compounds not available to plants uptake (Gaskin et al., 2010) rather than bio-available amine N (Cao and Harris, 2010; Chan and Xu, 2009; Novak et al., 2009).

From an agronomical point of view, biochar cannot be considered a considerable source of N, as concluded by Novak et al. (2009) who found that biochar had no evident effect either on total C or total combustible N (TCN) using pecan shell derived biochar incorporated to the soil up to a rate of 2%. However, Scheifele et al. (2014) proved that the labelled-biochar N was, at least in part, available to plants as demonstrated by the ^{15}N signature measured in the soybean tissues. On the other hand, biochar has the potential to improve the efficiency of exogenous N sources derived from fertilizers (Ding et al., 2010; Steiner et al., 2008; Gaskin et al., 2008; Chan et al., 2007).

During pyrolysis N is progressively volatilized resulting in a biochar with a C/N ratio much higher (> 30) than in the feedstock (Atkinson et al., 2010). Thereby, when incorporated into the soil, the labile C may stimulate the N requirement of bacteria and fungus to build new biomass. This will exhaust the N resources in the soil since microbes are more competitive than plant roots. The N immobilization in the microbial biomass after biochar addition represents a mechanism contributing to improve N retention in the topsoil (Sohi et al., 2010). However, if biochar is stable (high temperatures during pyrolysis), available C substrate is negligible, limiting microbial demand for external N. Whether significant N immobilization in soil occur depends on the rate of biochar addition, the consistence of the C labile fraction added and the N availability from either native (i.e. OM mineralization) or external sources (e.g. fertilizers).

Although mechanisms are not fully understood, literature reports that biochar alters the N dynamic in soil (Clough and Condron, 2010; Lehmann, 2007 and literature therein). For instance, biochar alone did not increase radish yield even at high rate (100 t ha^{-1}); however, increasing biochar application rates (10, 50 and 100 t ha^{-1}) significantly increased yield in combination with 100 kg ha^{-1} of mineral N (Chan et al., 2007a). The biochar used in the former study was characterized by a low N content (1.8 g kg^{-1}), a negligible mineral N fractions and a high C:N ratio, thereby it was suggested that its application did not contribute to any additional available N to the crop, but increased the N use efficiency. It has been proposed that biochar in soil has the ability to: (a) retain N by reducing ammonia (NH_3^+) production and promoting NH_4^+ bonding, (b) reduce nitrous oxide (N_2O) emission and NO_3^- leaching, and (c) enhance biological N fixation and benefit soil microbial communities (Clough and Condron, 2010; Asada et al., 2006).

Phosphorus

Biochar-induced increase in available P in soil has been suggested as one of the possible explanations for the positive response of plant growth and yield, in particular in tropical soils,

which are often acids, highly weathered and rich in sesquioxides (Van Wambeke, 1992) that bound phosphate (Turner et al., 2006), resulting in P deficiency for plants (Oberson et al., 2006). Reported changes on P cycle in soil include the transformation of stable P into more plant-available forms (Braker and Conrad, 2011; Glaser et al., 2002) and the addition of available biochar-associated P. For instance, Angst and Sohi (2012) concluded that provision of soil P by biochar from hardwood might be sustained for several seasons whereas K release declined rapidly. In a field study on a ferrosol soil, an increase in plant available P upon a manure-based biochar amendment but not upon greenwaste biochar was observed (Slavich et al., 2013). On the contrary, a small but statistically significant reduction in plant available P resulted following high biochar application rates (4.4% and 11%, w/w) to a sandy soil (Van Zwieten et al., 2010c). A mechanism based on the dehydration of phosphate by biochar has also been proposed (Beaton et al., 1960) as a possible explanation of an improved P uptake by plants upon biochar addition, possibly aided by arbuscular MF (Lehmann and Rondon, 2006).

Potassium

Biochar is often characterized by high concentration of exchangeable K (Chan et al., 2007a) as long as low-medium temperatures are reached through pyrolysis since K volatilizes at around 750°C, thereby most of the original content in the biomass is retained in the final product. As a result, available K content in soils has been observed to quickly increase after biochar application (Alling et al., 2014; Chan et al., 2007).

Calcium

Similarly to K, increase in exchangeable Ca in soil has been found following the application of biochar (Chan et al., 2007). An overall increase in available Ca from 101% to 320% was also reported in a long-term (4 year) field trial where biochar was applied at rates of 8 and 20 t ha^{-1}, respectively. Such increase was partially explained as the reduced Ca leaching induced by biochar application (Major et al. 2010a).

Magnesium

Increasing in soil available Mg upon the addition of biochar is explained by the direct release of this element contained in charred biomasses. The addition of 1, 5, and 10% of biochar provided 50, 250, and 500 µg of Mg, respectively (Alling et al., 2014). Major et al. (2010a) found that available Mg concentration in soil increased up to 217% after 4 years of a sole 20 t ha^{-1} biochar application.

Sulphur

There are not many evidences about the effect of biochar application on S content in soil. However, Novak et al. (2009) showed that exchangeable S slightly decreased with an increase of biochar addition obtained from pecan shells.

Micronutrients

During biochar formation, iron (Fe) and Mn are largely retained and associated under a number of organic and inorganic forms in the biomass (Amonette and Joseph 2009), thereby redistributed into chemical forms less soluble (Wang et al., 2009). However, Novak et al. (2009) measured an increased Mn concentration in soil after two months from the incorporation of a pecan shell based biochar but a significantly lower concentration of Mn was recorded in the leachate. Similarly, hardwood-derived biochar (22.4 t ha^{-1}) increased the available soil Mn and total organic C (TOC) by 1.5 and 1.4-fold in a calcareous soil, respectively (Lentz and Ippolito, 2011). Gaskin et al. (2008) describe a great variety in Zn concentration in biochars, depending on the feedstock used, while extractable Zn marginally decreased from 13 to 10 mg kg^{-1} with an increase in the addition of biochar to soil (Novak et al., 2009). Cu availability in soil was not significantly affected by the addition of biochar up to a rate of 20 g kg^{-1} of soil (Novak et al., 2009) but a notable decreases in leaf Cu, Fe, Mn and Zn concentration was observed on mustard, barley and red clover grown successively within one year after the incorporation of three different biochars in combination with 3 different soils (Kloss et al., 2014).

2.2.2.2 Influence of biochar on nutrient leaching in soil

Mineral leaching from agricultural lands implies agronomical, economic and environmental considerations since it depletes soil fertility, increases the amount of required synthetic and/or organic fertilizer inputs (< fertilizer use efficiency) and leads to eutrophication of water bodies contributing to lower the quality of ground and surface waters (Laird et al., 2010b; Sharpley et al., 2001). Furthermore, leaching in soil affects nutrient cycling in agriculture (Brady and Weil, 2008) and occurs when mobile ions dissolved in soil solution (not retained by colloids) move outside the rooting zone where plants cannot uptake them (Major et al., 2009). Water percolation depends on the soil infiltration capacity (hydraulic conductivity), water retention on the root zone and crop transpiration rate, which is related to the density and the ability of the roots to absorb water. In addition, amount, chemical form, timing and placement of synthetic

and/or organic fertilizers, greatly affect nutrient leaching patterns (van Es et al., 2002; Cahn et al., 1993).

Although biochar application may result in an initial increased in nutrient leaching (e.g., NO_3), especially when biochars have high N content (Singh et al., 2010), it has been proved that the application of charred biomasses is an effective, long-term tool for reducing the adverse impact of mineral leaching on surface and groundwater quality (Laird et al., 2010; Steiner et al., 2008; Lehmann et al., 2003). Based on the literature, biochar results effective in reducing the leaching of many ions (at least in the short-term) including PO_3^-, NH_4^+ and NO_3^-, which are usually the most limiting nutrients to crop growth (Lehmann et al., 2003). Biochar added to a manure treated soils reduced the total inorganic N mineral forms (NO_3 and NH_4) amount in the leachate by 11% in comparison with the soil added only with manure (Laird et al., 2010), while NO_3^-, but not NH_4^+ concentration in the leachate, was significantly reduced in an apple orchard amended with a biochar from hardwood (Ventura et al., 2013). In agreement with the former findings, a recent study suggests a role of biochar in retaining mineral N mostly in the form of NO_3^- rather than NH_4^+. In fact, the application of 15 and 30 t ha^{-1} of biochar in a sandy soil significantly increased NO_3^- concentration in the top soil (0-0.15 m) while it was decreased in the deeper layers (up to 0.90 m) (Kammann et al., 2014). Similarly, biochar at 30 and 60 t ha^{-1} reduced NO_3^- leaching by roughly 60% in a macrocosm study with *Vitis vinifera* grown in sandy soil (Kammann et al., 2014). The same authors revealed that both fresh and aged biochars (2%) sorbed up to 60% of the soil-applied labelled ^{15}N-NO_3^-. Depending on the biochar type (feedstock and charring T), soil characteristics and contact period, high biochar application rates (10 and 20 % biochar:soil w w^{-1}) have been shown to reduce NH_4^+ leaching in contrasting (Ferralsol and Anthrosol) soils (Lehmann et al., 2003). Leaching of applied NH_4^+ was reduced by more than 60% compared to unamended soil in cropping rice, whilst leaching of Ca and Mg was also reduced, by 20 and 40%, respectively, after 250 mm of applied water, but only during the first week (Lehmann et al., 2003). The same author did not report any effect on the leaching of K which was not reduced likely because fresh biochar typically contains large amounts of this element. The adsorbed N may be subsequently available to plants (Taghizadeh-Toosi et al., 2012; 2011), as shown by Chan et al. (2008) who observed an increase in the uptake of N at high rates of biochar.

Pepperwood biochar effectively reduced the amount of NO_3^--N, NH_4^+-N, and phosphate in the leachate of a sandy soil in a column study by 34.0%, 34.7%, and 20.6%, respectively, while a peanut hull biochar also reduced the leaching of NO_3^--N and NH_4^+-N by 34% and 14%, respectively, but caused an additional phosphate release from the soil columns (Yao et al.,

2012b), suggesting that the effect of biochar on the nutrient leaching of agricultural lands varies by biochar and nutrient type. In fact, the multiple interactions occurring within the soil-plant-atmosphere system (e.g. rainfall patterns, agricultural management practices) and the range of feedstocks (and their mixtures) potentially employable for biochar production make specific effects of biochar, hardly predictable as a generic qualities of biochar.

Benefits from a reduced leaching in agriculture are both direct (> fertilizer use efficiency) and indirect, considering that considerable fossil energy is required to fix N into fertilizers; thereby a low ratio of N application to crops and an increase in N uptake impact the overall C balance of agricultural activities and lead to a lower fertilizer requirement per unit yield, thus to lower N_2O emissions.

Different mechanisms have been associated to the decrease in nutrient leaching (or nutrient retention) when biochar is added to soils and these can be mainly ascribed to:

a) the great surface area of biochar provided with adsorption sites for inorganic nutrients (bound by ion and covalent bindings);
b) the increase in soil WHC which improves nutrient retention residence time in the root zone;
c) the increase of the internal reactive surface area of the soil-biochar matrix;
d) the decrease of water percolation below the root zone induced by an increased evaporative surface (plant water use);
e) the increase in plant nutrient use through enhanced crop growth;
f) the attachment of OM with sorbed nutrients onto biochar particles.

Biochar is a high porous material and its bulk density is lower than that of mineral soil, suggesting that its application modify soil hydrology because of changes in porosity and, in the long term, aggregation. A large percentage of the biochar pores are smaller than $<2 \times 10^{-3}$ μm (Tseng and Tseng, 2006), contributing to reduce the water mobility through the soil since water moves better in pores in the order of a few tens of micrometers in size (e.g. 30 μm) (Brady and Weil, 2008). Once incorporated, biochar can modify soil pore-size distribution (soil porosity) thereby alter percolation and flow patterns (Major et al., 2009). Soil porosity is critical in determining the rate at which rain can infiltrate into soil and carry nutrients away from the rooting zone. Altering soil porosity and consequently soil water content, biochar may reduce nutrient leaching, with a greater effectiveness in coarse-textured (sandy) than in silty or clayey soils, since evidences suggest that sandy soils amended with biochar will experience an increase in water content while the effect could be opposite in clay soil (Tryon, 1948).

Nevertheless, a reduced amount of leachate was recorded from a clayey soil mixed with biochar (Lehmann et al., 2003) and this response was attributed to the increased plant biomass and evaporative surfaces induced by biochar which indirectly reduced water mobility, while in sandy soils this mechanism can be complemented by the direct retention of water by biochar (Major et al., 2009). In addition, in sandy soil, where the amount of water held decrease as matric potential increases, biochar particles may hold large volumes of immobile water, even at elevated matric potentials. Thereby, biochar can contribute to retain minerals by trapping water held by capillary forces, as it occurs in soil micropores. Nutrients dissolved in this water would thus be retained near the soil surface and plants can access part of these nutrients (Major et al., 2009). A delay in the $N\text{-}NH_4^+$ concentration in the leachate was experienced in the top 0.10 m of a multi-layered soil column when soil was mixed with bamboo charcoal (biochar) at a rate of 5 g kg^{-1} and ammonium chloride (NH_4Cl) at 400 kg N ha^{-1} let to percolate (Ding et al. 2010). Authors attributed such response to the highly porous structure of biochar and its adsorptive properties.

Leaching reduction potential of biochar can be affected by particle size, since larger-sized biochar fragments generally sorb fewer nutrients than smaller ones, suggesting an effect of the total surface area (Major et al., 2009).

Compared to larger biochar fragments, the smaller ones, as well as small negatively charged soil colloids (Sen and Khilar, 2006), can facilitate the physical transport of the retained nutrients through the soil profile, since small particles may travel downwards with water percolation and/or horizontally by surface water runoff (Major, et al., 2010b). Soil particles up to 10 μm were found to move downward through a sandy loam soil (Jacobsen et al., 1997) while particles with a size between 2 and 5 μm moved from topsoil through a sandy loam in the field (Laubel et al., 1999) and natural colloids (up to 200 μm) were mobilized through a coarse soil (Totsche et al., 2007). Size of biochar fragments dramatically varies upon the same biochar batch and very small particles (e.g. <2 μm: the size of clay particles) can represent a large proportion of the produced material (after pyrolysis) or created during transport and incorporation into the soil. Furthermore, physical (e.g. pounding, tillage, rain, water freezing and thawing), chemical weathering and biotic disturbance continuously participate in reducing biochar particles size resulting in finer biochar over time, suggesting that the ability of biochar to reduce leaching could also decrease over time.

N fluxes in soil include denitrification and gaseous losses (i.e. N_2O), fixation, precipitation, immobilization, mineralization and, also, leaching which in turn represent the N cycle. Each of these components can be altered whether directly or indirectly by biochar in terms of rate and

time course, thus affecting consequently rate and time course of the leachate. For instance, affecting N_2O emission (see paragraph 2.2.1.7 for details), biochar alters the amount of N in soil, which may undergo to different fates. Similarly, biochar application to agricultural lands may led to an increased net nitrification rate (Gundale and DeLuca, 2007; Berglund et al., 2004), mostly due to the sorption of nitrification-inhibiting phenolic compounds which affect the availability of the nitric N form, hence the potential amount of the mineral forms leachable. Also, the addition of biochar has also been suggested to improve microbial growth (Rondon et al., 2007) with possible implications in the nitrification and denitrification processes. Inhibition and increase in the nitrification activity in soils upon the addition of biochar has been proposed (Clough and Condron, 2010; DeLuca et al., 2006; DeLuca and Sala, 2006). However, mechanisms about how nitrifying bacteria can be affected by biochar in soil are not fully understood.

Furthermore, leaching rate is affected by soil texture (clays in particular), soil minerals and OM as well as the chemistry of the elements in the soil solution. Whether a nutrient is under organic or inorganic form, size and charge properties of the molecules determine how it interacts with other particles of the soil matrix. In fact, positively charged ions or molecules (e.g. NH_4^+, Ca^{++}, Fe^{++}), can be retained by negatively charged clays and soil OM (Brady and Weil, 2008) particles. Similarly, negatively charged ions e.g. (NO_3^-) can be retained by positively charged compounds. These properties are quantified as cation (CEC) and anion (AEC) exchange capacity and refer to the ability of a substance to retain positively or negatively charged ions, respectively. Biochars produced through slow pyrolysis between 250 and 900 °C are characterized by negatively charged sites on its surface, a high surface area and large internal porosity and by the presence of both polar and nonpolar surface sites (Mukherjee et al., 2011; Novak et al., 2009; Baldock and Smernik, 2002; Glaser et al., 2002). For this reasons, biochar has often a CEC consistently higher than that of whole soil, clay minerals or soil OM, typically ranging between 30 and 150 cmolc kg^{-1} which makes it able to sorb and desorb positively charged nutrients (Liang et al., 2006) through electrostatic forces, while AEC of biochar is often very low and, therefore, the adsorption of anions (i.e. NO_3^- and $PO_4^{=}$) is quite negligible or absent (Hale et al., 2013; Yao et al., 2012b; Braker and Conrad, 2011). Yao et al. (2012b) reported a weak NH_4^+ adsorption by the majority of 13 biochars, which adsorbed between 1.8 and 15.7% of the added amount of NH_4^+.

Higher nutrient retention and nutrient availability after charcoal addition were observed by Glaser et al. (2002) who concluded that charcoal contributed to an increase in ion retention and to a decrease in leaching of dissolved OM and organic nutrients in acidic tropical soils. Acidic

soils, in fact, often ensure a low CEC due to the abundance of H^+ ions which occupy available sites, hence a biochar-induced pH increase is beneficial also to increase nutrient retention and it has been indicated as one of the likely reasons for observed increase in crop yields upon its application (Atkinson et al., 2010).

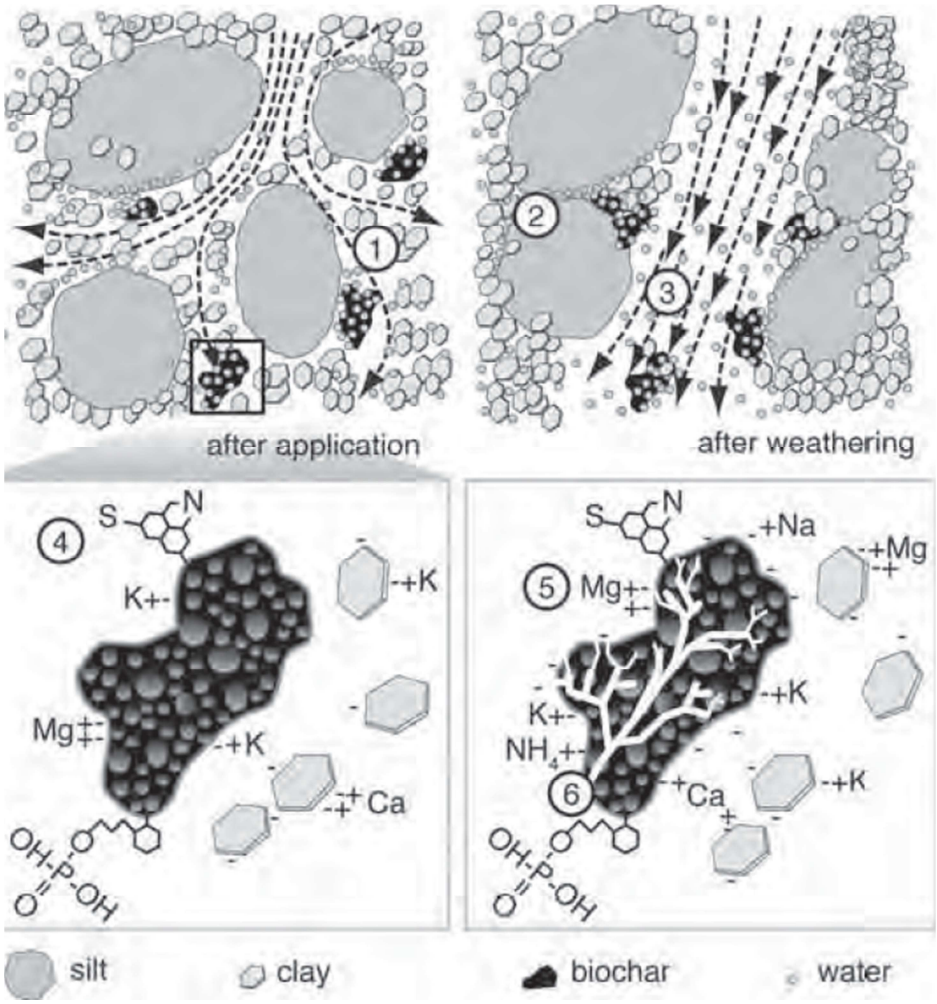

Figure 2.3. Proposed biochar effects on nutrient leaching: 1) Upon biochar application, soil WHC increases due to water reduced mobility as a consequence of biochar porosity; 2) after weathering, as biochar binds to other soil constituents, soil aggregation is improved, and 3) preferential water flows occur as well as the smoothed transport of biochar particles; 4) at a smaller scale, newly applied biochar sorbs hydrophobic organic forms of nutrients and 5) after weathering, the surface charge of biochar increases, thus improving cation exchange capacity, and 6) promoting soil biota.

(Source: Major et al., 2009, with permission)

Illustration is not strictly to scale and water is not shown in the bottom panels.

Furthermore, nutrient adsorption through charge or covalent interaction on biochar is promoted by biochar porosity which led to a large surface area to which both hydrophobic and hydrophilic molecules can be adsorbed (Major et al., 2009) depending upon the functional groups displayed by the biochar. Major et al. (2012) showed that biochar produced at temperatures above 500°C (or stem activated), resulted in an increased surface area and thus in an increased sorption of nutrients. Functional groups found on biochar surfaces have been indicated as the responsible for the interactions with water and solutes. These include hydroxyl (-OH), carbonyl (C=O), carboxylate (COOH), hydrogen (H) and ether (R–O–R) (Mao et al., 2012; Cheng et al., 2008) which influence biochar surface chemistry by Coulombic, dipole and H-bonding reactions. Carboxylate groups are primarily responsible for the CEC of biochar due to their negative formal charges, while others functional groups on biochar are polar nano-sites on a relatively non-polar and hydrophobic surface (Lawrinenko, 2014). This surface structure can sorb molecules with both polar and non-polar portions. Besides, O-contained alcohol, carbonyl, and carboxylate functional groups are generally believed to contribute to biochar CEC because they may carry a negative charge and serve as Lewis bases for the sorption of cations. Finally, O and N containing chemical functional groups in biochar contribute to surface properties as they are generally polar and provide sites for hydrogen bonding, ion-dipole, and dipole-dipole interactions (Lawrinenko, 2014).

2.3 Heavy metals supply and remediation associated with biochar application

The supply of biochar to lands has the potential to contaminate the environment as a consequence of heavy metals (Bridle and Pritchard, 2004) and harmful compounds, such as polycyclic aromatic hydrocarbons (PAHs), that may be condensed on its surface during pyrolysis (Fabbri et al., 2013; Joseph et al., 2010). PAHs refer to fused aromatic rings and are generally part of oil, coal, and tar deposits as well as by-products of burning of fossil fuel or biomass and these compounds are of main concerns due to their carcinogenic, mutagenic, and teratogenic nature (Kookana et al., 2011). The potential contamination risk associated with biochar supply is more accentuated when municipal wastes, sewage sludge, industrial wastes, chicken litter and compost are used as feedstocks to produce biochar (Verheijen et al., 2010). Moreover, due to its high sorption capacity (Laird, 2008), biochar may favor the accumulation of persistent organic pollution (POPs) in soil.

Bridle and Pritchard, (2004) reported a high heavy metal content for biochar obtained from different feedstocks and in particular, high concentration of Cu, Zn, Cr and Ni were detected in

a biochar produced from sewage sludges. However, biochar obtained from the former biomass did not induce heavy metals accumulation in soil after its incorporation (Shinogi et al., 2003)

On the other hand, it is well known that activated carbon-based products, due to their unique properties, especially their highly carbonaceous and aromatic nature and high specific surface area, are strong adsorbents of micronutrients, toxic metals (Ogbonnaya and Semple, 2013; Huang, 2003; 1978; Budinova et al., 1994) and polar compounds including many environmental contaminants (i.e. PAH, dioxin) (Hale et al., 2012; Hilber et al., 2012). Cao et al. (2009) concluded that biochar produced from dairy manure is a greater sorbent to remove lead (Pb) and atrazine from soil than activated carbon, while increase in the sorption of benzene and toluene onto red gum charcoal was reported by Bornemann et al. (2007). The former outcome is supported by Chen and Yuan (2011), who noticed a positive decontamination result induced by pine needle biochar incorporated to a soil spiked with naphthalene, phenanthrene or pyrene. Mohan et al. (2007) studied the adsorption potential for arsenic (As), cadmium (Cd) and Pb of oak bark, pine bark, oak wood and pine wood chars obtained by fast pyrolysis up to 450 °C and a commercial activated charcoal; oak bark char adsorbed maximum Pb, Cd, and As and authors indicated the high CEC as the main mechanism for such response. Broiler litter manure char (350 and 700 °C) and their steam-activated analogues adsorbed heavy metals in the sequence Ni < Cd < Cu < Pb from a mixture containing these metals (Uchimiya et al., 2010). As, Cd, and Cu concentration was decreased in maize shoots grown in a contaminated soil whereas the effect was inconsistent for Pb and Zn (Namgay et al., 2010) and this response was attributed to the sorption of the metal(loid)s by biochar.

Application of biochar significantly reduced soil NH_4NO_3 extractable Cd (cadmium), Cu and Pb concentration with consequent reduced accumulation of these metals by Indian mustard (*Brassica juncea*) tissues. Authors suggested that these metals were immobilized and that biochar modified the partitioning of Cd, Cu and Pb from the easily exchangeable phase to less bioavailable organic bound fraction (Park et al., 2011).

Beesley et al. (2010) proved that biochar was effective in reducing the concentration of the phytotoxic water soluble Cd and Zn and total and bio-available PAHs in a multi-element contaminated soil. In the same study, toxic Cu and Pb concentrations were significantly reduced while the effect of biochar on the uptake of As into Miscanthus foliage (Hartley et al., 2009) was negligible. Other studies proved the capacity of biochar to remove dissolved NO_3^- (Mizuta et al., 2004) and phosphate (Beaton et al., 1960). More recently, Oleszczuk et al. (2012) reported that addition of either biochar or activated C can mitigate the mass transfer of

contaminants from PAH-containing sewage sludge matrix into pore-water. Likewise, biochar in soil may reduce the bioavailability of pesticides since it has been reported to be >2000 times more effective than soil in sorbing these compounds, reducing their plant availability at relatively small rate (0.05% by wt) (Kookana, 2010).

The sorptive capacity of biochar could also be used to remove contaminants during wastewater treatment process. For instance, the pine chars was successfully used to treat a fluoride-contaminated groundwater at pH 2.0. Ion exchange and metal fluoride precipitation were addicted as the main mechanisms of adsorption (Mohan et al., 2012).

Compared to larger biochar particles or to particulate OM, biochar dust has been indicated as a better sorbent for a wide range of trace hydrophobic contaminants in soils (e.g. PAHs, polychlorinated biphenyls - PCBs, pesticides, polychlorinated dibenzeno-p-dioxins and –furans - PCDD/PCDFs) (Hiller et al., 2007; Bucheli and Gustafsson, 2001, 2003) as well as in marine system (i.e. dioxin) (Persson et al., 2002).

2.4 Impact of biochar on soil fertility and crop production

Terra preta de Indio is evidently more fertile than surrounding lands and this was generally attributed to its higher proportion of black C (Lehmann and Rondon, 2006; Glaser et al., 2002; Haumaier et al., 1995), likely originated from partially-combusted biomass residues derived from a range of anthropogenic activities such as kitchen fires and field burning. In these soils, differences in crop productivity were strongly associated with soil CEC (Liang et al., 2006; Glaser et al., 2002), suggesting that charcoal positively affected nutrient availability in plant-available form and cations retention, minimizing leaching losses. Other studies report that the liming effect of charcoal was the main factor for improved crop yields on acidic soils (Verheijen, et al., 2010). However, while water and nutrient retention are expected to continue over time upon biochar application, nutrient supply and liming effect are supposed to last shorter. Positive responses on crop yield have been also attributed to the direct addition of available plant nutrients such as P, K, Ca, Zn and Cu and a consequent increased plant uptake in tropical environments (Alburquerque et al., 2013; Lehmann and Rondon, 2006). Moreover, charcoal may also contain bioavailable elements (e.g. selenium (Se)) that could potentially assist crop growth (Sohi et al., 2009).

To date, published studies assessing the effect of anthropogenic soil-applied biochar on crop yield are generally short-term and limited to small experimental sites, often carried out in pots where environmental fluctuation is limited. Nevertheless, evidences suggest that biochar application to soil at moderate rates (0.5 t ha^{-1}) are usually beneficial (Glaser, 2002) and in few

cases negative, at least for some crops or soils (Gaskin et al., 2010; Van Zwieten, et al., 2010a; Sohi et al., 2009) while high rates seem to inhibit plant growth. Higher rates than 0.5 t ha^{-1}, along with chemical NPK source, increased crop yield on tropical Amazonian soils (Steiner et al., 2007) and semi-arid soils in Australia (Ogawa, 2006). In addition, positive responses of crop yields have been documented in pot and field trials (Alburquerque et al., 2013; Huang et al., 2013; Spokas et al., 2012; Chen et al., 2010; Major et al., 2010; Van Zwieten, et al., 2010a; Asai et al., 2009; Chan et al., 2008; 2007).

Kammann et al. (2012) observed a significant increase in the biomass of ryegrass (*Lolium perenne* L.) after the addition of peanut (*Arachis hypogaea* L.) hull biochar at a rate of 50 t ha^{-1} to a German Luvisol soil. Major et al. (2010) attributed the increased yield of maize to a 77-320% greater availability of Ca and Mg in the biochar amended soil while reduced nutrient losses was indicated as the main factor for increased crop yield on infertile sandy soils (Asai et al., 2009; Steiner et al., 2008). Chan et al. (2007) concluded that the addition of biochar along with fertilizers significantly increased radish yields more than the addition of fertilizer alone, indicating the increased N use efficiency as the key factor.

Plant dry biomass increased by 353 and 572% for shoot and root, respectively after the addition of 10 g kg^{-1} of a chicken manure-derived biochar and this response was attributed to a reduced toxicity of metals and increased availability of nutrients such as P and K (Park et al., 2011). An increase up to 200% is reported in the rice yield (Noguera et al., 2010) using charcoal (wood) at rate of 5 g kg^{-1} (biochar:soil) in a lab experiment. In a cropping trial (*Vigna unguiculata* and *O. sativa*) carried out in an archaeological Anthrosol soil, a significantly increased of P, Ca, Mn and Zn availability was indicated as the responsible for the increase (38–45%) in biomass production of the two crops in the biochar amended plots (Lehmann et al. 2003). In a *Zea mays* trial carried out in Western Kenya (Kimetu et al., 2008), the application of biochar doubled crop yield, although this response was not explained by biochar nutrient availability alone. A yield increase of *R. sativus* was observed after the application of 10, 25 and 50 t ha^{-1} of poultry manure biochar alone (Chan et al., 2008). However, crop yield response after biochar addition is not always positive (Spokas et al., 2012). In fact, crop yield response after biochar addition could be positive, neutral or even detrimental (Mukherjee and Lal, 2014; Crane-Droesch et al., 2013; Spokas et al., 2012 and literature therein). For instance, a reduced growth in wheat and radish with the addition of a paper mill sludge biochar in a calcarosol soil was reported by Van Zwieten et al. (2010a). Similarly, a 30% decrease in the biomass of *R. sativus* grown on an Alfisol soil after the incorporation of 10 t ha^{-1} green waste biochar was observed, although biomass increased at higher biochar rates (Waters et al., 2011). Other

studies have reported a decline in soil N availability with wood biochar addition, potentially causing reduced yields (Asai et al. 2009). The amendment of biochar alone (8 t ha^{-1}) or biochar mixed with compost (8 + 55 t ha^{-1}, respectively) in a 30-year-old vineyard induced economically irrelevant and mostly non-significant effects on yield and grapevine quality over three years field trial (Schmidt et al., 2014).

In conclusion, data extracted from Jeffery et al. (2011), who resumed the relationship between biochar and crop productivity using the meta-analyses approach, show an overall relatively small, but statistically significant, positive effect of biochar application to soils on crop production (approximately 10%). In the same study, the greatest responses were seen in 39% of included trials when biochar was applied at 100 t ha^{-1}. Positive effects were recorded mainly in acid (14%) and neutral pH soils (13%), and in soils with a coarse or medium texture (10 and 13%, respectively), suggesting that two of the main mechanisms for yield improvement may be a liming effect and the influence on the WHC. In agreement with the previous study, a recent meta-regression analysis estimated an average crop yield increase of approximately 10% for 3 Mg ha^{-1} biochar addition in the first year after application (Crane-Droesch et al., 2013). In the latter study, soil properties (low cation exchange capacity and low organic C content) showed the best predictability with positive yield response while in contrast with previous findings soil pH and soil clay content were not significantly correlated to increased yield response.

Interestingly, prediction models about potential benefits induced by the addition of biochar to agricultural soils implicate positive yield response over much of Sub-Saharan Africa, parts of South America, Southeast Asia and southeastern North America (areas of highly weathered soils in tropics or subtropics) and the north of Eastern Europe. Yield response is predicted to be mostly negative in organic soils such as those of Indonesia, northern Eurasia and North America, while yield response

may be weak or negative in many of the most important grain-producing areas, such as the Eurasian chernozems, central North American mollisols, South Asian vertisols soils and in large areas of the North American corn belt (Crane-Dresch et al., 2013).

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CHAPTER 3

Research interests and aims

Due to its potential to mitigate climate changes and benefit both soil fertility and crop yield, biochar has worldwide increasingly attracted, in the last 10 years, the interest of scientists, politicians, entrepreneurs, growers, media and public opinion as proven by the steady increasing number of peer-reviewed papers, international conferences, granted projects, voluntary initiatives as well as enterprises involved in biochar production and/or trading (IBI, 2014).

Production and subsequent incorporation of biochar, especially into temperate soils, is a novel approach for establishing a long-term sink for atmospheric CO_2 storage and achieving agronomic benefits (Atkinson et al., 2010). Although promising, this approach involves economic, environmental and agronomic implications which are only beginning to be explored, thereby it must be scientifically investigated before adopted by growers.

Even though it is quite accepted that biochar in soil interacts with microbes, plant roots, water and minerals, the extent, rates and implications of these interactions are not fully understood. Scientific understanding about biochar effects and mechanisms in soils as well as the long-term environmental exposure on biochar properties are still lacking (Joseph et al., 2010). Furthermore, most of the scientific evidences on biochar as a soil conditioner were obtained in tropical and subtropical environments, in acid, weathered and scarcely fertile soils (Jeffery et al., 2011), thus proper evidences of the environmental impact and mechanisms of soil-applied biochar on perennial crops grown in the Mediterranean basin are required. Evidences are frequently limited to few years after biochar application since most of the results are referred to annual crops and, often, grown in controlled environment, therefore the long-term effect on perennial crops in field conditions has been poorly studied.

The objective of this project was to evaluate the effect of biochar on perennial crops in terms of agronomical, biological and environmental impacts.

Specific objectives were to:

- ✓ Study the interaction between biochar and minerals in solution;
- ✓ Investigate the effect of biochar on Fe nutrition in perennial plants;
- ✓ Estimate the effect of biochar and compost on soil leaching and nutrient losses;
- ✓ Evaluate the effect of biochar and compost on soil CO_2 flux partitioning and fertility;

- ✓ Characterize soil bacterial biodiversity and key gene expression of soil nitrification-involved bacteria as affected by biochar in combination with or without compost;
- ✓ Compare increasing rates of biochar on plant growth, nutritional status, yield and fruit quality of nectarine trees grown in field conditions;
- ✓ Evaluate the long-term environmental exposure on biochar physical-chemical changes.

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CHAPTER 4

Biochar interferes with kiwifruit Fe-nutrition in calcareous soil

Abstract

The effect of soil-applied biochar on lime-induced iron (Fe)-deficiency on susceptible kiwifruit trees was investigated. Results from a 2-year pot experiment demonstrate that biochar significantly reduced soil-extractable micronutrients (Fe, manganese (Mn), copper (Cu) and zinc (Zn)) and worsened Fe-chlorosis incidence on kiwifruit plants, likely as a consequence of a reduced Fe availability. Then, the effectiveness of soil-applied aqueous extract of *A. retroflexus* (alone and enriched with $FeSO_4$) was explored, because of its ability to extract Fe from soil by Fe-chelating compounds released from its tissues and synthetic siderite (Fe(II) carbonate ($FeCO_3$) as sustainable strategies to improve Fe nutrition of kiwifruit trees grown in calcareous soil. Furthermore, the potential of biochar to release and retain micronutrients in solution was also investigated. In addition, chemical surface changes of biochar fragments exposed to a Fe source by X-ray photoelectron spectroscopy (XPS) technique was assessed.

The aqueous extract of *A. retroflexus* enriched with $FeSO_4$ and siderite were effective in alleviating Fe chlorosis symptoms of kiwifruit trees. Biochar had little value as a source of micronutrients but retained large amounts of Fe, Mn, Cu and Zn dissolved in solution, likely through reactive functional groups on its surface. Diffused rusty spots were evident on the biochar surface after its exposure to a Fe source and a redox reaction between biochar and the Fe is one possible explanation.

Keywords: Micronutrients, soil extractable Fe, redox reaction, siderite, *A. retroflexus* aqueous extract, $FeSO_4$.

4.1 INTRODUCTION

Biochar is produced by pyrolysis of biomass, typically at temperatures below 700°C (Atkinsons et al., 2010). This C-rich material, used as a soil conditioner, has been proposed as a potential strategy to mitigate climate change and benefit both soil fertility and crop yield (Laird, 2008; Lehmann, 2007 and references therein). Incorporation of biochar, especially into temperate soils, is a novel approach for establishing a long-term sink for atmospheric carbon dioxide (CO_2) storage and agronomic benefits (Atkinson et al., 2010), but while it is widely accepted that biochar interacts with microbes, plant roots, water and minerals in soils, extensive understanding about mechanisms of such interactions are not fully understood (Joseph et al., 2010). For instance, interactions between biochar and specific micronutrients in different conditions and their impact on plant nutrition have not been investigated yet.

Recently, it has been suggested that biochar can take up and release several hundred micromoles of electrons (Klüpfel et al., 2014) suggesting that biochar could potentially affect biogeochemical cycles in soil, not only by changing soil physic and chemical properties, but also by mediating electron transfer processes (i.e., as an electron shuttle) altering, among others, the iron (Fe) cycle in soils (Kappler et al., 2014).

So far, most of the scientific evidences on the use of biochar as a soil conditioner come from tropical and subtropical environments characterized by acidic, weathered and scarcely fertile soils (Jeffery et al., 2011). Few observations are reported about the effect of biochar addition on perennial crops grown in the Mediterranean areas, where soils are often calcareous with high pH, as it characterizes approximately 39% of world soils (Çelik and Katkat, 2010). Consequently, although promising, the biochar approach involves economic, environmental and agronomic implications which must be scientifically investigated before widely adopted.

Lime-induced Fe-chlorosis is a widespread nutritional disorder (Pic. 4.1) occurring on both susceptible perennial and annual crops when grown in calcareous and alkaline soils (Abadía et al., 2011; Pestana et al., 2003). In these soils, as a consequence of the high pH and the active lime fraction, Fe in solution precipitates

as scarcely soluble Fe-hydroxides (Röemheld and Nikolic, 2007). In addition, the enzymatic activity of the root Fe^{III}-chelate reductase, responsible for the reduction of the Fe^{III} to Fe^{II}, is dramatically compromised (Susin et al., 1996), thereby limiting the availability of the ionic Fe form absorbable by roots of dicots (Röemheld and Marschner, 1986). Fe-deficiency negatively affects leaf chlorophyll (Chl) concentration (Abadía and Abadía, 1993) and implies disturbances in leaf water relations (Eichert et al., 2010; Fernández et al., 2008). As a consequence, light absorption, photosystem II and Rubisco carboxylation efficiencies in chlorotic leaves are depressed (Larbi et al., 2006). Limited Fe availability for plant uptake might heavily prejudice yield and fruit quality (Sorrenti et al., 2012; Álvarez-Fernández et al., 2006 and literature therein), decrease tree vigor and shorten orchard productive lifetime of several species, including kiwifruit which is considered among the most susceptible crops to this disorder (Tagliavini and Rombolà, 2001).

Picture 4.1. Severe symptoms of Fe-chlorosis on a kiwifruit orchard (left) and detail of a leaf

Although effective (Abadía et al., 2011; Lucena, 2006), either soil- or foliar-applied synthetic Fe-chelates to prevent or curing Fe-chlorosis induce a short-lasting re-greening effect and pose economic (Tagliavini and Rombolà, 2001) and environmental concerns (Grčman et al., 2001; Nörtemann, 1999) since they can be easily leached. The development of cost-effective and environmental friendly strategies to overcome Fe-chlorosis is of interest (Pestana et al., 2003).

Recently, it has been described that the aqueous vegetal extract of *Amaranthus retroflexus* increased significantly the amount of Fe extracted from the soil compared to deionized water (d-H_2O) and that the supply of the same extract in fertigation improved Fe-nutrition of pear trees (Sorrenti et al., 2011). This response was attributed to natural Fe chelating compounds released by the *Amaranthus* spp. tissues (Matocha, 1984) according to the concept of "Plant-Complexed-Fe" (Matocha and Pennington, 1982). On the other hand, the slow release of Fe from soil-applied synthetic minerals such as vivianite ($Fe_3(PO_4)_2*8H_2O$) and siderite (a Fe(II) carbonate ($FeCO_3$), achieved a long-lasting prevention of Fe-chlorosis in different crops (Cañasveras et al., 2014; Rosado et al., 2002; Sánchez-Alcalá et al., 2012a, b). Reacting with soil carbonate, these minerals produce poorly crystalline Fe oxides (Sánchez-Alcalá et al. 2012a; Roldán et al., 2002), which are considered to be the main source of Fe to plants (de Santiago and Delgado, 2006).

The aims of this study were: i) investigate the effectiveness of soil-applied sustainable strategies (aqueous extract of *A. retroflexus* and synthetic siderite) and their interaction with biochar in preventing Fe-chlorosis of kiwifruit grown in calcareous soil, ii) evaluate the ability of biochar to act as a source of micronutrients, iii) assess the potential of biochar in retaining micronutrients in solution iv) characterize the chemical biochar surface changes after the exposure to a Fe source.

4.2 MATERIALS AND METHODS

4.2.1 Interaction between biochar and Fe nutrition on kiwifruit

4.2.1.1 Experimental design and growth conditions

We performed a 2-year experiment (2011-12) outdoors at the experimental station of the University of Bologna (in Cadriano, Bologna, 44°55' N, 11°40' E, 36 m a.s.l.) on 1-year old micropropagated kiwifruit (*Actinidia deliciosa* cv Hayward) grown in 5.2 L pots filled with a heavy alkaline-calcareous soil (Tab. 4.1). Trees were trained as in a single shoot, watered daily (in summer) by microirrigation to return the evapotranspiration (ETo) rate as estimated by a class A evaporation Pan and the specific crop coefficient (Kc) for kiwifruit and covered with a shade netting which allowed a light intensity of 73.500 lux (measured in summer at noon on a

sunny day). Except for Fe, tree nutrient requirements (nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg)) were satisfied by periodic supply of a nutrient solution by fertigation and weeds were manually removed from pots.

The experiment was arranged in a complete randomized factorial experimental design with 6 replicates (single tree) and 2 factors: fertilization (5 levels) and biochar (2 levels). We compared the following fertilization treatments: a) untreated control; b) commercial Fe-chelate (1.0 g L^{-1} of commercial Fe-EDDHA with a Fe content of 6% in the ortho-ortho isomer); c) synthetic siderite (1.0 g of suspension per kg of soil); d) aqueous extract of *A. retroflexus* at a rate of 30 g (dw) L^{-1}, and e) aqueous extract of *A. retroflexus* at a rate of 30 g (dw) L^{-1} mixed with Fe(II) sulphate heptahydrate ($FeSO_4*7H_2O$) at a rate of 2 g L^{-1}. Treatments were either applied to: i) unamended soil or ii) soil amended with biochar at a rate of 50 g fw kg^{-1} (w w^{-1}) equal to 52 t fw ha^{-1} (considering a treated area of 2 m wide along tree row in a commercial kiwifruit orchard with a 3*5 m spacing (667 tree ha^{-1}), soil incorporation up to 0.20 m depth and a specific soil weight of 1.3 t m^{-3}).

The biochar we used in this experiment consisted of small chunks obtained from a mixed feedstock of fruit trees pruning wood, with a prevalence of peach (*Prunus persica* L.) and grapevine (*Vitis vinifera* L.) pyrolized at approximately 500°C at atmospheric pressure. Biochar physical and chemical characteristics are summarized in table 1.

The aqueous extract was prepared and characterized as described by Sorrenti et al. (2011). Briefly, the dried powder of *A. retroflexus* was macerated in tap water (pH 7.4; Fe < 0.08 mg L^{-1}) at least 24 h before its application, maintaining the suspension at room temperature and in the dark. Each treatment was applied at weekly intervals 4 and 5 times in the first and second season, respectively, starting from bud burst and at a rate of 200 mL $plant^{-1}$.

Synthetic siderite was prepared by mixing 40 g L^{-1} of potassium carbonate (K_2CO_3) (Sigma-Aldrich) to a pot containing 80 g L^{-1} of Fe(II) sulphate-heptahydrate ($FeSO_4*7H_2O$) which provided a brownish green suspension containing 34 g L^{-1} of siderite. The solution was continuously stirred and the suspension was mixed to the soil only once at planting at a rate of 125 mL per pot in order to apply 1 g of siderite per kg of soil.

At the same time as the aqueous vegetal extract applications, Fe-chelate treated plants received 200 mL $plant^{-1}$ of Fe-chelate solution while untreated and siderite treated plants received the same amount of tap water.

4.2.1.2 Fe chlorosis incidence, tree nutritional status and plant biomass

We estimated the incidence of Fe-chlorosis periodically by the determination of the leaf Chl concentration of the first apical completely expanded leaf per shoot measured by a hand-held Chl meter (SPAD 502, Minolta Co. LTD, Osaka, Japan). Shoot length was also periodically recorded. In July of the second season, we collected random fully expanded leaves per tree, petioles were removed then leaf laminas were washed in a 0.1 N HCl solution supplemented with a surfactant (Tween 20) at a rate of 1 mL L^{-1}, as recommended by Álvarez-Fernàndez et al. (2001), rinsed abundantly in d-H_2O, oven-dried (65 °C) and milled (0.2 mm mesh). We determined leaf macro (N, P, K, calcium (Ca), Mg) and micro (Fe, manganese (Mn), zinc (Zn) and copper (Cu)) nutrient concentration. Total N concentration was determined by the Kjeldahl method (Schuman et al., 1973) by mineralizing 1.5 g of sample with 12 mL of a 95:5 (v v^{-1}) H_2SO_4:H_3PO_3 mixture, at 420 °C, for 180 min and subsequent distillation with 32% (v v^{-1}) NaOH and titration with 0.2 M HCl. Phosphorus was spectrophotometrically quantified at 700 nm, through extract mineralization (Saunders and Williams, 1955) of 0.5 g of tissue with 96% (v v^{-1}) sulphuric acid and 35% (v v^{-1}) oxygen peroxide, and subsequent neutralization with 0.1 M NaOH enriched with 0.1 M ascorbic acid, 32 mM ammonium molybdate, 2.5 M sulphuric acid and 3 mM potassium antimonyl tartrate to develop a phospho-molybdic blue color. Metal concentrations were determined by atomic absorption spectrophotometry (AAS) (Varian AA200, Mulgrave, Victoria, Australia) after wet digestion according to US EPA Method 3052 (Kingston, 1988). To this end, 0.5 g of dry matter were mineralized in an Ethos TC microwave labstation (Milestone, Bergamo, Italy) by adding 8 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%) at 180 °C. Lanthanum chloride ($LaCl_3$ at 10%) and caesium chloride (CsCl at 5%) solutions were added to the samples at ratios of 20% and 4%, respectively prior to K, Ca and Mg readings. At the end of the second season,

trees were harvested and divided into shoots and roots (including stem), oven-dried and weighted.

4.2.1.3 Soil pH and soil extractable micronutrients

At the end of the experiment, a soil sample (1 kg) was collected from each pot, oven-dried (105°C), ground (1 mm mesh) and used to determine soil pH and soil diethylenetriamine-pentaacetic-acid (DTPA) (Lindsay and Norwell, 1978) extractable Fe, Mn, Cu and Zn concentration. We also determined soil extractable Fe using d-H_2O as eluent. The DTPA solution was obtained by mixing 0.005 M DTPA, 0.01 M calcium chloride ($CaCl_2$) 0.1 M and triethanolamine, then the pH was adjusted to 7.3 ± 0.05 by 5 M HCl. For both eluents (d-H_2O and DTPA), eighty mL were added to 40 g (dw) of soil, shaken 2 h, let to decant then filtrated (Whatmann, 41). The micronutrient concentration in solution was determined by AAS (Varian AA200, Mulgrave, Victoria, Australia). Soil pH was determined in a soil:d-H_2O solution at a rate of 1:2.5 (w w^{-1}). 10 g of soil were added to 25 mL of d-H_2O, then solutions were stirred 1 h prior readings under continuous stirring by pH meter (Crison, pH- Meter BasiC 20, Barcelona, Spain).

4.2.2 Biochar micronutrients release and retention potential

We used the same biochar batch described in the previous experiment. However, biochar was first sieved to remove ash and impurity and to homogenize the size of the fragments that ranged between 2 and 7.5 mm. Three replicates of biochar were repeatedly washed to reduce ash and tar content by adding 4 L of d-H_2O to 200 g of biochar and shacking 30 min at 100 rpm by an orbital shaker. At the end of every washing, we collected the supernatant that was analysed for electrical conductivity (CE) (Crison, Conductivity meter 524, Barcelona, Spain). Washing steps were repeated (7 times) until constant CE, which started from 661.3 ± 8.83 µS (avg. ± SE n=3) after the first washing and ended at 51.5 ± 1.64 µS (avg. ± SE n=3). The washed biochar was then oven-dried at 30°C. After this, 25 mL of d-H_2O were added to glass flasks containing 4, 10, 20, 30 and 40 g L^{-1} of biochar, with 5 replicates. Four series of flasks, with same rates of biochar, were added with solutions containing 10 mg L^{-1} of one of the following cations: Fe, Mn, Zn or Cu.

Pure $FeCL_3*6H_2O$, $MnCl_2*4H_2O$, $ZnCl_2$ and $CuSO_4$ diluted in HCl 0.12 N standard solutions were used as a source of micronutrients. The pH of the solutions was adjusted by sodium hydroxide (NaOH) at 7.2 ± 0.1, then flasks were shacked on an orbital shaker for 120 min at 90 rpm. The supernatant was filtrated (Wathmann 42) and analyzed for micronutrients by AAS (Varian AA200, Mulgrave, Victoria, Australia).

4.2.3 Biochar surface chemistry change as affected by Fe source exposure

We added three medium size washed biochar fragments, as above mentioned, to 25 ml of d-H_2O or to a solution made of d-H_2O added with 100 mg of Fe file dust (> 350 µm) obtained from a commercial Fe bar, with 3 replicates. Fragments were maintained in solution 1 week then removed and oven-dried at 65°C. One fragment per replicate was analyzed by XPS to determine whether or not the char surface chemistry was altered as a consequence of the exposure to the Fe source. The top 5 nm biochar surface was analyzed for relative Fe, O and C atomic concentration (at%) using a PHI Quantera XPS with an Al X-ray source at 1486.6 eV and 49.2 W. The beam diameter was 200.0 µm and the pass energy 26 eV. XPS spectra were analyzed using a nonlinear least-squares curve-fitting program with a Gaussian−Lorentzian mixed function to optimize the spectra which were analyzed using MultiPak data analysis software (MultiPak V7.0.1, 04 Mar 16, Ulvac-Phi, Inc., 1994-2004).

4.3 STATISTICAL ANALYSES

Pot experiment data were evaluated by analysis of variance according to a complete randomized factorial design with 2 factors: fertilization (5 levels) and biochar (2 levels) with 6 replicates. When analysis of variance showed a statistical effect (at $P \leq 0.05$), means were separated by Student-Newman-Keuls Test (SNK); when interaction between fertilization treatment and biochar was significant, 2 times standard error of means (SEM) was used as the minimum difference between two statistically different means (Saville and Rowarth, 2008). Coefficient of determination (R^2) between biochar rate and micronutrients concentration was calculated in the lab experiment. Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC).

4.4 RESULTS

4.4.1 Interaction between biochar and Fe nutrition on kiwifruit

4.4.1.1 Fe chlorosis incidence, tree nutritional status and plant biomass

Pronounced leaf Fe chlorosis symptoms appeared both seasons in untreated trees (as estimated by the lowest values of leaf Chl content (SPAD units) and, except for the aqueous extract of *A. retroflexus* alone, all strategies were effective in significantly increasing leaf SPAD content (Tab. 4.3 and 4.4), without interaction between fertilization strategy and biochar.

Soil-applied Fe-chelate always induced the highest SPAD index (Tab. 4.3 and 4.4). Siderite and *A. retroflexus* enriched with $FeSO_4$ showed SPAD values similar to Fe-chelate, particularly in the first season of investigation, whereas *A. retroflexus* alone was ineffective in preventing Fe chlorosis occurrence (Tab. 4.4). Leaf Fe-deficiency symptoms were significantly evident in plants grown in biochar amended soil, but only in the first season, though (Tab. 4.3 and 4.4).

All control and aqueous extract *A. retroflexus*-alone treated trees, independently of the presence of biochar in soil, died in summer of the second season.

In 2011, shoot length was significantly increased by the aqueous extract of *A. retroflexus* enriched with $FeSO_4$ compared to siderite in the first measurement and to the other treatments in the second assessment (Tab. 4.5). In 2012 shoot growth was promoted by Fe-chelate and, to a less extent, by *A. retroflexus* enriched and siderite compared to control plants (Tab. 4.5). However, at the end of the second season, no significant differences were observed among the remaining strategies (Tab. 4.5). Independently of the fertilization, shoot growth was significantly depressed in Sep-2011 and Jun-2012 by the presence of biochar in soil, without interaction between factors (Tab. 4.5).

At the end of the experiment, Fe-chelate and *A. retroflexus* enriched with $FeSO_4$ significantly promoted shoot and root (including stem) dry weight, respectively compared with siderite (Tab. 4.6). However, no statistical differences were recorded between the highest values (Tab. 4.6). As anticipated, the 12 untreated as well as the 12 *A. retroflexus* alone treated plants died in summer of the second season, thereby no shoots were sampled at plant harvest. In addition, a poorly developed root system (including stem) was recovered from these pots (Tab. 4.6).

Picture 4.2. Effect of the fertilization strategy in combination with biochar on plant growth at the end of the experiment

Fe-chelate and *A. retroflexus* + $FeSO_4$ resulted in a higher total plant biomass compared to other strategies (Tab. 4.6). The addition of biochar to the soil did not affect organs neither total plant biomass, without interaction between factors (Tab. 4.6).

Leaf K concentration was decreased in plants treated with Fe-chelate compared to other treatments while a significant interaction between treatment and biochar occurred for Mg, Fe and Mn (Tab. 4.7 and 4.8). When soil was amended with biochar leaf Mg and Fe concentrations were significantly decreased in plants treated with Fe-chelate and the aqueous extract enriched with $FeSO_4$, but not by siderite (Tab. 4.7 and 4.8). Only in unamended soil leaf Mg concentration was significantly increased by Fe-chelate compared to the aqueous extract enriched and siderite (Tab. 4.8). The supply of Fe-chelate dramatically increased leaf Fe and decreased leaf Mn concentration compared to other treatments, independently of the substrate (Tab. 4.8). Similarly, leaf Fe concentration resulted higher in plants

fertilized with the suspension of siderite in comparison to those fertigated with the aqueous extract enriched (Tab. 4.8). The same trend was observed for leaf Mn, although it increased only in the biochar amended soil (Tab. 4.8).

Independently of the fertilization strategy, biochar induced an increase in leaf K and a reduction in leaf Ca concentration (Tab. 4.7 and 4.8), while no effects were observed on leaf N, P, Cu and Zn concentration (Tab. 4.7 and 4.8).

4.4.1.2 Soil pH and soil extractable micronutrients

At the end of the experiment, no interaction between biochar and fertilization strategies was observed on soil pH, which resulted significantly higher in soil enriched with biochar (Tab. 4.9). Among fertilizers soil pH was decreased by Fe chelate, *A. retroflexus* enriched and siderite (Tab. 4.9).

When d-H_2O was used as eluent, only synthetic Fe treatment significantly increased soil extractable Fe (Tab. 4.9), while when DTPA was used, the *A. retroflexus* + $FeSO_4$ induced the highest value of extracted Fe, followed by siderite (Tab. 4.9). The latter increased DTPA extractable Fe compared to soils untreated and fertigated with the vegetal extract alone, while intermediate values were obtained by Fe-chelate (Tab. 4.9). Compared to other strategies, soil DTPA extractable Cu was reduced by Fe-chelate application whereas soil DTPA extractable Mn and Zn were not affected by fertilization treatments (Tab. 4.10).

Soil enriched with biochar significantly decreased the amount of Fe extracted by d-H_2O while an opposite trend emerged using DTPA as extractor agent (Tab. 4.9). A similar response was also measured for soil DTPA extractable Mn, Cu and Zn concentration (Tab. 4.10), without interaction with fertilization strategies (Tab. 4.10).

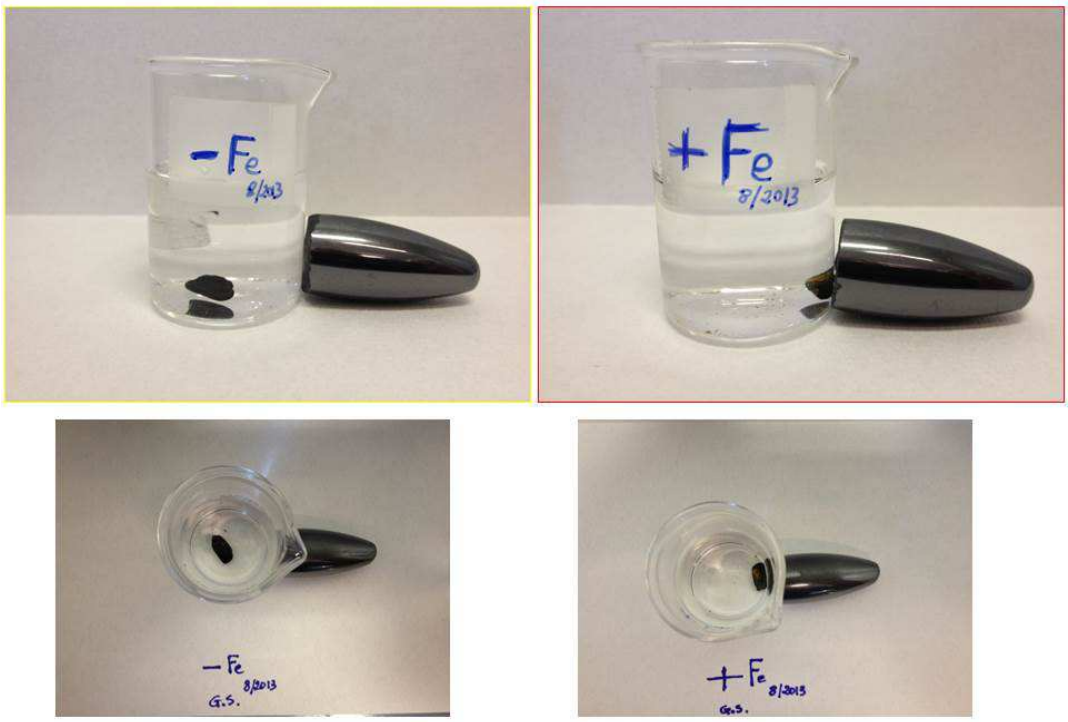
4.4.2 Biochar micronutrients release and retention potential

Independently of the rate, the amount of Fe, Mn, Cu and Zn released by biochar in d-H_2O was negligible (data not shown). Values of these metals in solution were lower than 0.015 mg L^{-1} in average, thus comparable to pure d-H_2O. Therefore, no significant correlation was obtained between biochar rate and released micronutrient concentration (data not shown).

Conversely, when biochar was dipped in 10 mg L^{-1} solutions of Fe, Mn, Cu and Zn (separately), it significantly reduced the amount of cations in solution as the biochar rate increased (Fig. 4.1). The concentration of dissolved Fe after the addition of biochar was significantly correlated with biochar rate by a polynomial trend and R^2 was 0.92 (Fig. 4.1). Similarly, Mn, Cu and Zn concentrations were inversely and linearly correlated with biochar rate and R^2 values were 0.93, 0.93 and 0.88, respectively (Fig. 4.1).

4.4.3 Biochar surface chemistry change as affected by Fe source exposure

After the exposure to the Fe source (Pic. 4.3), biochar fragments showed diffuse rusty spots distributed over their surface (Fig. 4.2) and the surface relative atomic concentration, as measured by XPS, was 14.9 ± 2.14, 65 ± 5.21 and 22.6 ± 7.36 (at%, avg. ± SE) for Fe, O and C, respectively, (Fig. 4.3). On the contrary, biochar surface dipped 1 week in d-H_2O was not visibly altered in colour and its surface Fe atomic concentration resulted in 0.12±0.002, 14.2±1.62 and 86.1±2.60 for Fe, O and C, respectively, (Fig. 4.3).



Picture 4.3. After the exposure to an Fe source, the biochar fragment was attracted by a magnet

4.5 DISCUSSION

4.5.1 Sustainable strategies were effective in preventing Fe-chlorosis

Typical leaf Fe-chlorosis symptoms (interveinal yellowing starting from youngest leaves) appeared in untreated plants beginning in the first growing season, as a consequence of a limited plant Fe availability induced by the heavy calcareous soil. These symptoms were effectively prevented in both seasons by soil-applied Fe-chelate, which induced the highest SPAD values. Symptoms were prevented to a similar extent by the *A. retroflexus* aqueous extract (only when enriched with $FeSO_4$) and siderite in the first year, while the latter two treatments were slightly less effective the following year. Untreated and *A. retroflexus* aqueous extract alone treated plants died as a consequence of the persistent Fe deficiency induced by the extremely prohibitive growing conditions, confirming the high susceptibility of kiwifruit plants to this disorder and the severe consequences of Fe-chlorosis.

The beneficial effect on Fe nutrition induced by *A. retroflexus* aqueous extract is likely attributable to the chelating compounds (e.g. organic acids, aminoacids, bioregulator-like substances, siderophores) released by the *A. retroflexus spp.* tissues during either maceration or soil-incorporation (Matocha, 1984; Matocha and Pennington, 1982; Mostaghimi and Matocha, 1988; Goos et al., 2001) and, in fact, Sorrenti et al. (2011) measured a 100-fold higher concentration of solubilised Fe from a similar calcareous soil using the aqueous vegetal extract of *A. retroflexus* as Fe-extractant compared to d-H_2O. In our conditions the *A. retroflexus* aqueous extract alone did not improve Fe nutrition likely because the amount of solubilized Fe from a soil naturally poor in Fe was insufficient to sustain plant Fe requirements while it was enough when the aqueous extract was enriched with an exogenous source of Fe which promoted a weak linkage between Fe and the organic compounds released by plant tissues. A similar response was observed in field conditions (Sorrenti et al., 2011), where Fe nutrition of pear trees was improved by the vegetal extract only when enriched with $FeSO_4$, suggesting a beneficial effect of the *A. retroflexus* as a natural chelator since its Fe concentration is negligible.

We did not provide a treatment based on the addition of soil-applied $FeSO_4$ alone because it is already known that the supply of inorganic Fe-salts is inefficient in high pH (e.g. alkaline-calcareous) soils in enhancing Fe availability due to the

rapid transformation of most of the applied Fe into highly insoluble compounds such as Fe(III)-hydroxides (Tagliavini et al., 2000).

Siderite has been proposed as a long-term slow-release Fe fertilizer able to prevent Fe chlorosis symptoms in olive trees (Sánchez-Alcalá et al., 2012a). As for synthetic vivianite, the effectiveness of siderite is due to its oxidation in calcareous medium resulting into poorly crystalline lepidocrocite and/or goethite (Sánchez-Alcalá et al., 2012b) that, as with other poorly crystalline of nanometric size Fe oxides, represent a significant source of Fe for plants (Sánchez-Alcalá et al., 2012b; de Santiago and Delgado, 2006). However, the application of siderite (only once at the beginning of the experiment) was more effective in the first than in the second season suggesting that its effectiveness may last shorter than in previous studies (Sánchez-Alcalá et al., 2012a and literature therein).

The increased leaf K concentration observed in plant treated with siderite and vegetal extract of *A. retroflexus* could be a consequence of the direct supply of this nutrient by siderite (obtained by mixing KCO_3 and $FeSO_4$), while the vegetal extract may have a possible positive effect also on extracting this macronutrient from soil. A similar increase in leaf K concentration was observed in pear trees fertigated with *A. retroflexus* aqueous extract (Sorrenti et al., 2011).

In agreement with literature, Fe-chelate treatments dramatically decreased leaf Mn concentration because of the competitive effect of these synthetic molecules on Mn uptake as shown in herbaceous as well as in perennial species (Sorrenti et al. 2011; Wallace and Alexander 1973; Ghasemi Fasaei et al. 2003;) and suggesting that, to avoid Mn-deficiency, supplementary applications to the canopy of this micronutrient should be considered in commercial orchards when Fe-chelates are yearly used.

4.5.2 Biochar hinders kiwifruit Fe nutrition

Independently of the fertilization strategy, leaf Chl values were significantly reduced (in the first season) in amended compared with unamended soil; similarly, a shoot growth reduction was observed in some cases both seasons, suggesting that the presence of biochar in soil reduces Fe uptake. On the other hand, while biochar positively affected leaf K concentration as a consequence of the considerable

release of this nutrient in solution (data not shown), it significantly reduced leaf Fe accumulation in Fe-chelate and *A. retroflexus* enriched with $FeSO_4$-treated plants. In the latter treatment we also recorded a significant reduction of leaf Mn concentration induced by biochar. The pH increase (less than 0.1) in amended soil in part explains the negative effect of biochar on plant Fe and Mn uptake. Nevertheless, a significantly lower Fe concentration in soil solution (-63%) was extracted by d-H_2O when biochar was present while this concentration was enhanced by using a stronger extractant (DTPA). A similar trend was also observed for the other micronutrients when using DTPA suggesting that biochar retained Fe (as well as other cations) from soil and a week eluent, such as d-H_20, was ineffective to solubilise it. On the other hand, a reduction of the Mn^{2+} concentration was also measured in 6 out of 8 soils upon the addition of biochar (Alling et al., 2014). Therefore, we hypothesize that in potted conditions Fe in soil solution was attracted and retained by biochar (as observed in the second experiment), thereby limiting its availability (in particular in the first season) for plant uptake, accentuating Fe-chlorosis symptoms of kiwifruit trees. After the first season, symptoms of Fe-chlorosis in biochar amended trees were less evident suggesting that the negative effect does not last long. This is probably because the biochar surface after the first season was, at least in part, saturated with cations or because a sort of cation exchange equilibrium was reached, leading to an increase of Fe availability for plant uptake. The fact that Fe was attracted by biochar in soil has been reported also by Lin et al. (2012) who, using transmission electron microscopy (TEM) equipped with energy dispersive spectroscopy (EDS) for elemental analysis, showed that the mineral matter attached to the biochar surfaces on fragments recovered after a 3-month soil incubation experiment included higher concentration of elements such as O, Al, Si, Fe, Ti and trace amounts of other elements such as Mn, Mg, Ca, K, Na, P, and S compared to fresh biochars.

4.5.3 Micronutrient release from biochar is negligible

Although it has been suggested that biochar may represent a potential source of macro- and micronutrients for plants and the soil microbial community (Downie et al., 2009), direct nutrient supply by biochar is often negligible, in particular when

vegetal biomass is used as feedstock (Singh et al., 2010; Gaskin et al., 2008). Nutrient content and chemical structure differ greatly among biochars and are strongly influenced by the pyrolysis conditions and secondarily by feedstock (Kookana et al., 2011, Singh et al., 2010; Gaskin et al., 2008). In addition, nutrient content and availability frequently decrease with increasing pyrolysis temperature. Usually, nutrient release by biochar is related to its ash content which solubilization may result in readily-available nutrients to plants. In our conditions, independently of the rate, biochar did not affect Fe, Mn, Cu and Zn concentration in solution (data not shown). This response was expected and could be ascribed: i) to the pyrolyzed feedstock made of hardwood characterized by a poor nutrient content, ii) to the absence of ash, since biochar was repeatedly washed prior test and iii) because micronutrients (i.e. Fe and Mn) in biochar are considered to be largely retained under a number of organic and inorganic forms during biochar formation (Amonette and Joseph 2009), thereby redistributed into chemical forms less soluble (Wang et al., 2009), confirming the scarce value of biochar as a direct source of micronutrients (Novak et al., 2009).

4.5.4 Biochar acts as a retaining additive for micronutrients in soil

Conversely, biochar showed a high retention potential for micronutrients when available in solution. In fact, the addition of increasing rates of biochar, thereby total surface area, reduced progressively the amount of the initial content of all micronutrients in solution (Fig. 4.1), suggesting that biochar attracted and retained cations as demonstrated also by other studies (Novak et al., 2009). The affinity of biochar for micronutrients was in the order Fe>Cu>Zn>Mn with a retention of 100% of the initial Fe and Cu content (10 mg L^{-1}) with a biochar rate between 20 and 30 and 30 and 40 g L^{-1}, respectively. The retention of Zn and Mn was ~80 and ~50% of the initial content, respectively with the highest biochar rate (40 g L^{-1}).

The ability of biochar to sorb and desorb nutrients has been ascribed to its cation exchange capacity (CEC) and/or anion exchange capacity (AEC) (Liang et al., 2006). CEC measures the ability of a substrate to retain positively charged ions (e.g. NH_4^+, Ca^{++}, Fe^{++}) through electrostatic forces, while AEC refers to the retention of negatively charged ions (e.g. NO_3^-). Functional groups found on

biochar surfaces have been indicated as the responsible for the interactions with water and solutes. These include hydroxyl (-OH), carbonyl (C=O), carboxylate (COOH), hydrogen (H) and ether (R–O–R) (Cheng et al., 2008; Mao et al., 2012) which influence biochar surface chemistry by Coulombic, dipole and H-bonding reactions. Carboxylate groups are primarily responsible for the CEC of biochar due to their negative formal charges while others functional groups on biochar are polar nano-sites on a relatively non-polar and hydrophobic surface (Lawrinenko, 2014). This surface structure can sorb molecules with both polar and non-polar portions. Besides, O containing alcohol, carbonyl, and carboxylate functional groups are generally believed to contribute to biochar CEC because their negative charge as they serve as Lewis bases for the sorption of cations. Finally, O and N containing chemical functional groups in biochar contribute to surface properties as they are generally polar and provide sites for hydrogen bonding, ion-dipole, and dipole-dipole interactions (Lawrinenko, 2014).

The ability of our biochar to retain cations could have been accentuated by the fact that our biochar was 4-year old. In fact, it has been suggested that the CEC of biochar increase with biochar ages as shown by high concentrations of negative charges on biochar surface due to surface oxidation induced by abiotic processes (Cheng et al, 2006), while AEC is likely to be reduced.

Therefore, another possible explanation to the observed retention effect could be attributed to a physic mechanism due to the high surface area and porosity of biochar which increase the contact between biochar particles and solution (Major et al., 2009). The amount of water hold by biochar in our test was not taken into account, thereby nutrient sorption might partly result from dissolved nutrients physically held in the porous structure of biochar before drying and analysis. However, water absorption cannot completely explain the observed differences since doubtful 100% of Fe and Cu amount in solution was physically trapped into biochar pores.

4.5.5 Fe exposure induces a redox reaction on biochar surface

Recently, it has been demonstrated that since biochar is redox-active (Klüpfel et al., 2014), it can be involved in abiotic redox reactions (Oh et al., 2013) and, depending on the feedstock and charring temperature, biochar can take up (accept) and release (donate) several hundred micromoles of electrons per gram (Klüpfel et al., 2014). Moreover, results from Kappler et al. (2014) suggest that biochar in soil can alter soil biogeochemistry either indirectly by changing the soil structure and chemistry or directly by mediating electron transfer processes, by functioning as an electron shuttle. In our experiment biochar clearly showed diffuse rusty spots on its surface after the exposure to the Fe source and the relative surface atomic concentration significantly changed for Fe, O and C. The relative O and Fe concentration of biochar surface was much higher than in control fragments, suggesting that Fe was oxidized on biochar surface. Because Fe metal particles (used as a source of Fe) were bigger in size than biochar pores, it is unlikely that they were physically trapped on biochar surface. Although other reactions cannot be excluded, we hypothesize that the Fe source interacted with the biochar surface and a redox reaction occurred. The Fe source released Fe^{2+} in solution that was electrostatically attracted to the biochar surface (Kappler et al., 2014) by reactive carboxylic and phenolic functional groups (Lin et al., 2012). The same biochar was the donor of electrons (other than water) originating the following redox reaction:

$$Fe \rightarrow Fe^{2+} + 2\ e^{-} \rightarrow Fe^{3+} + 1\ e^{-}\ \text{(oxidation)}$$

$$O_2 + 4\ e^{-} + 2\ H_2O \rightarrow 4\ OH^{-}\ \text{(reduction);}$$

the product of such reaction can be summarized as follow:

$$4Fe + 3O_2 + 6H_2O \rightarrow\ 4Fe(OH)_3$$

Then, the ferric hydroxide ($Fe(OH)_3$) precipitated as hydrated ferric oxides, originating rust as final product:

$$2Fe(OH)_3 \rightarrow Fe_2O_3 \cdot 3H_2O$$

4.6 CONCLUSIONS

Biochar showed a high potential to remove Fe, Mn, Cu and Zn dissolved in solution, likely through reactive functional groups on its surface. On the other hand it showed little value as a source of these micronutrients. The affinity of biochar for cations together with the fact that it can accept and donate electrons could trigger redox reactions in soil with significant implications for biogeochemical cycles, thereby also affecting nutrient forms and availability for plants. In our conditions, we speculate that biochar in soil sequestered part of the available micronutrients (in particular Fe), limiting their availability for plant uptake. Incorporating biochar in Fe-limited growing environments (i.e. alkaline-calcareous soils) hindered plant Fe nutrition, worsening the Fe-chlorosis occurrence on kiwifruit trees. This response should be taken into consideration in the development of biochar as an agronomic technique and adequate countermeasures need to be evaluated. However, future studies are needed to confirm this effect in different soils and how aging will affect these properties of biochar.

Finally, our results indicate the potential of innovative and sustainable strategies (aqueous extract of *A. retroflexus* enriched with $FeSO_4$ and siderite) to alleviate symptoms of Fe chlorosis, improving Fe nutrition of kiwifruit trees grown in heavy calcareous soil.

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Table 4.1. Selected chemical and physical characteristics of the soil used in the experiment

Parameter	**Unit**	**Value**	**Extractant/method**
Texture			
Sand	%	24	
Lime	%	53	
Clay	%	23	
Total carbonate ($CaCO_3$)	%	78	HCl / De Astis method
Active lime ($CaCO_3$)	%	19.2	Ammonium oxalate (Drouineau, 1942)
Organic matter	%	0.54	Walkley-Black 1919 (Soltner, 1988)
Total N	‰	0.39	Kjeldahl method
Assimilable phosphorus (P)	mg kg^{-1}	3	Olsen (Olsen and Sommers, 1982)
Exchangeable potassium (K)	mg kg^{-1}	195	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable sodium (Na)	mg kg^{-1}	186	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable calcium (Ca)	mg kg^{-1}	2611	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable magnesium (Mg)	mg kg^{-1}	47	Barium chloride (Hendershot and Duquette, 1986)
Assimilable iron (Fe)	mg kg^{-1}	2.68	DTPA (Soltanpour and Schwab, 1977)
Assimilable manganese (Mn)	mg kg^{-1}	1	DTPA (Soltanpour and Schwab, 1977)
Assimilable zinc (Zn)	mg kg^{-1}	0.51	DTPA (Soltanpour and Schwab, 1977)
Exchangeable copper (Cu)	mg kg^{-1}	2.21	DTPA (Soltanpour and Schwab, 1977)
Exchangeable Boron (B)	mg kg^{-1}	0.29	Calcium chloride (Bingham, 1982)
C/N ratio		8.03	
Cation Exch. Capacity (CEC)	meq $100g^{-1}$	14.7	Barium chloride (Hendershot and Duquette, 1986)
pH		8.39	Water/Potentiometric

Table 4.2. Physical and chemical characteristics of the biochar

Parameters	Unit	Value
Physical properties		
Moisture	%[1]	13.8
Bulk density	g cm^{-3}	0.43±0.04
Hydrophobicity		Slightly hydrophobic
Total porosity	mm^3 g^{-1}	2722
Transmission pores	mm^3 g^{-1}	318
Storage pores	mm^3 g^{-1}	1997
Residuals pores	mm^3 g^{-1}	406
Max water absorption	g g^{-1} of d.m.	4.53
Skeletal density (SD)[2]	g cm^{-3}	1.86±0.04
Envelope density (ED)[3]	g cm^{-3}	0.2459±0.0056
Porosity (ED/SD)	%	0.863±0.00574
Surface area[1] (BET Brunauer–Emmett–Teller method)	m^2 g^{-1}	410±6
Particle size distribution[1]	mm g^{-1}	
50-20	%	4.45
20-10	%	12.1
10-8	%	13.1
8-4	%	10.36
4-2	%	19.85
2-1	%	24.2
<1	%	15.94
Chemical properties		
pH	-	9.8
CEC	cmolc kg^{-1}	101
Carbon[1] (C)	g kg^{-1}	778.0
Total nitrogen (N)	g kg^{-1}	9.1
C/N	-	85.49
Aluminum (Al)	mg kg^{-1}	268
Arsenic (As)	mg kg^{-1}	0.005
Beryllium (Be)	mg kg^{-1}	0.001
Cadmium (Cd)	mg kg^{-1}	0.001
Calcium (Ca)	g kg^{-1}	25.0
Chrome (Cr)	mg kg^{-1}	0.002
Cobalt (Co)	mg kg^{-1}	0.002
Copper (Cu)	mg kg^{-1}	97
Iron (Fe)	mg kg^{-1}	333
Magnesium (Mg)	g kg^{-1}	28.7
Manganese (Mn)	mg kg^{-1}	84
Molybdenum (Mo)	mg kg^{-1}	2
Phosphorus (P)	g kg^{-1}	23.3
Potassium (K)	g kg^{-1}	13.9
Sodium (Na)	g kg^{-1}	11.9
Sulphur (S)	mg kg^{-1}	481
Zinc (Zn)	mg kg^{-1}	104

[1]data obtained from Baronti et al. (2014) (with permission). [2]The skeletal density is the sample mass divided by sample volume occupied by a solid sample, including any pores not accessible to the helium gas. [3]The envelope density is defined as the sample mass divided by the total sample volume that is measured if an "envelope" would be placed around each individual particle.

Table 4.3. Effect of the fertilization and biochar on leaf chlorophyll content in the first season

Fertilization	**Leaf Chlorophyll Content (Spad units)**					
	Apr-13	**May-20**	**Jun-24**	**Jul-25**	**Aug-26**	**Set-11**
Control	26.5	22.4 bc	18.9 b	20.2 b	19.6 b	18.3 b
Fe-chelate	27.4	30.3 a	29.5 a	34.4 a	30.0 a	28.6 a
Siderite	27.6	26.8 abc	34 a	23.9 a	33.5 a	33.0 a
A. retroflexus	28	21.2 c	15.5 b	20.7 b	18.2 b	14.7 b
A. retroflexus + $FeSO_4$	28.7	27.2 ab	28.6 a	30.0 a	29.3 a	28.5 a
Significance	ns	*	***	**	**	***
Biochar (g kg^{-1})						
0	27.6	29.2	29.3	31.8	30.6	28.7
50	27.7	21.8	21.0	21.3	20.3	21.8
Significance	ns	***	**	***	***	**
Fert x Biochar	ns[1]	ns	ns	ns	ns	ns

[1]ns, *, ** and *** = effect not significant or significant at P <0.05, P <0.01 and P <0.001, respectively.
When a significant effect occurred, means in the same column followed by the same letter are not statistically different (P <0.05, SNK Test).

Table 4.4. Effect of the fertilization and biochar on leaf chlorophyll content in the second season

Fertilization	**Leaf Chlorophyll Content (Spad units)**									
	May-14	**May-21**	**May-31**	**Jun-7**	**Jun-14**	**Jun-27**	**Jul-11**	**Aug-28**	**Sep-5**	**Sep-13**
Control	10.0 b	9.8 b	10.0 d	10.4 d	9.2 c	8.9 b	11.5 b	-	-	-
Fe-chelate	23.9 a	28.5 a	31.7 a	37.7 a	33.0 a	31.6 a	28.3 a	25.8	26.7	27.9 a
Siderite	25.1 a	27.0 a	19.4 c	19.4 c	20.7 b	27.8 a	29.0 a	26.4	24.8	20.3 b
A. retroflexus	11.8 b	13.7 b	15.3 c	15.8 c	11.1 c	10.8 b	9.65 b	-	-	-
A. retroflexus + $FeSO_4$	24.6 a	27.5 a	24.6 b	28.4 b	24.1 b	32.0 a	31.7 a	27.3	24.4	20.2 b
Significance	***	***	***	***	***	***	***	ns	ns	***
Biochar (g kg^{-1})										
0	20.1	21.3	21.9	23.4	19.9	23.2	26.7	26.6	26.9	22.5
50	20.1	23.8	19.8	23.3	24.4	25.1	28.3	26.5	24.1	23.8
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fert x Biochar	ns[1]	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]ns and *** = effect not significant or significant at P <0.001, respectively.
When a significant effect occurred, means in the same column followed by the same letter are not statistically different (P <0.05, SNK Test).

Table 4.5. Effect of the fertilization and biochar on shoot length

Fertilization	**Shoot Length (cm shoot^{-1})**						
	2011			**2012**			
	Jun-24	**Sep-25**	**May-21**	**Jun-7**	**Jun-27**	**Aug-8**	**Sep-13**
Control	36.0 ab	38.1 bc	16.0 c	20.5 b	24.7 d	-	-
Fe-chelate	37.6 ab	50.2 b	37.7 ab	74.2 a	120.2 a	47.2	61.8
Siderite	26.7 b	39.2 bc	21.1 bc	52.7 a	69.2 c	48.6	63.5
A. retroflexus	32.7 ab	31.1 c	18.0 c	31.4 b	33.9 d	-	-
A. retroflexus + $FeSO_4$	49.6 a	69.6 a	40.2 a	70.0 a	84.6 b	48	70.4
Significance	*	**	*	***	***	ns	ns
Biochar (g kg^{-1})							
0	39.5	51.0	26	48.9	84.6	50.1	68.3
50	33.5	40.3	25.5	55.9	63.6	45.3	62.2
Significance	ns	*	ns	ns	*	ns	ns
Fert x Biochar	ns[1]	ns	ns	ns	ns	ns	ns

[1]ns, *, ** and *** = effect not significant or significant at P <0.05, P <0.01 and P <0.001, respectively. When a significant effect occurred, means in the same column followed by the same letter are not statistically different (P <0.05, SNK Test).

Table 4.6. Effect of the fertilization and biochar on plant biomass at the end of the experiment

Fertilization	**Plant biomass at harvest**		
	Shoot	**Stem+ Root**	**Total Dry Biomass**
	(g dw plant^{-1})	**(g dw plant^{-1})**	**(g dw plant^{-1})**
Control	-[1]	13.1 c	13.1 c
Fe-chelate	18.2 a	50.6 ab	68.8 a
Siderite	12.2 b	42.2 b	54.4 b
A. retroflexus	-	11.2 c	11.2 c
A. retroflexus + $FeSO_4$	13.3 ab	51.3 a	64.6 a
Significance	*	***	***
Biochar (g kg^{-1})			
0	15.3	34.9	50.2
50	13.9	31.0	44.9
Significance	ns	ns	ns
Fert x Biochar	ns[2]	ns	ns

[1]At the end of the experiment, shoots of the control and *A. retroflexus* treated plants were not present because plants had died.

[2]ns, * and *** = effect not significant or significant at P <0.05, and P <0.001, respectively. When a significant effect occurred, means in the same column followed by the same letter are not statistically different (P <0.05, SNK Test).

Table 4.7. Effect of the fertilization and biochar on leaf macronutrient concentration in July 2012

Fertilization	**N**	**P**	**K**	**Ca**	**Mg**	
	g kg^{-1}	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	
					Biochar 0	**Biochar 5%**
Fe-chelate	19.93	2.34	15.16 b	20.03	5.48	3.82
Siderite	22.42	2.44	25.55 a	20.38	3.51	3.80
A. retroflexus + $FeSO_4$	19.73	2.15	23.29 a	20.64	4.37	3.52
Significance	ns	ns	***	ns	2SEM = 0.75	
Biochar (g kg^{-1})						
0	21.32	2.23	18.92	22.87		
50	19.61	2.40	23.74	17.83		
Significance	ns	ns	***	*		
Fert x Biochar	ns[1]	ns	ns	ns	*	

[1]ns, * and *** = effect not significant or significant at P <0.05, and P <0.001, respectively.
When a significant effect occurred, means in the same column followed by the same letter are not statistically different (P <0.05, SNK Test).
*: interaction between fertilization and biochar significant at P <0.05. Values differing by ≥ 2 standard error of means (SEM) are statistically different

Table 4.8. Effect of the fertilization and biochar on leaf micronutrient concentration in July 2012

Fertilization	**Fe**		**Mn**		**Cu**	**Zn**
	mg kg^{-1}		**mg kg^{-1}**		**mg kg^{-1}**	**mg kg^{-1}**
	Biochar 0	**Biochar 5%**	**Biochar 0**	**Biochar 5%**		
Fe-chelate	68.3	62.3	2.41	2.92	9.17	22.10
Siderite	48.8	49.4	13.33	14.45	8.62	22.31
A. retroflexus + $FeSO_4$	42.1	39.4	14.12	8.50	8.35	19.96
Significance	2SEM = 2.59		2SEM = 2.41		ns	ns
Biochar (g kg^{-1})						
0					9.03	22.24
50					8.39	20.67
Significance					ns	ns
Fert x Biochar	*[1]		**		ns	ns

[1]ns, * and **: effect not significant or interaction between fertilization and biochar significant at P <0.05 and P <0.01, respectively. Values differing by ≥ 2 standard error of means (SEM) are statistically different

Table 4.9. Effect of the fertilization and biochar on soil pH and soil deionized water (d-H_2O) and DTPA extractable Fe at the end of the experiment (September, 2012)

Fertilization	**Soil pH**	**Soil d-H_2O extractable Fe**	**Soil DTPA extractable Fe**
		mg kg^{-1} (dw)	mg kg^{-1} (dw)
Control	7.52 a	0.25 b	1.94 c
Fe-chelate	7.46 b	0.53 a	2.36 bc
Siderite	7.48 b	0.26 b	2.90 b
A. retroflexus	7.59 a	0.32 b	2.02 c
A. retroflexus + $FeSO_4$	7.45 b	0.20 b	3.84 a
Significance	**	**	***
Biochar (g kg^{-1})			
0	7.45	0.44	2.42
50	7.56	0.19	2.80
Significance	***	***	*
Fert x Biochar	ns[1]	ns	ns

[1]ns, *, ** and *** = effect not significant or significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. When a significant effect occurred, means in the same column followed by the same letter are not statistically different ($P < 0.05$, SNK Test).

Table 4.10. Effect of the fertilization and biochar on soil DTPA extractable Mn, Cu and Zn concentration

Fertilization	**Soil DTPA extractable Mn**	**Soil DTPA extractable Cu**	**Soil DTPA extractable Zn**
	mg kg^{-1} (dw)	mg kg^{-1} (dw)	mg kg^{-1} (dw)
Control	5.20	2.63 a	2.04
Fe-chelate	6.22	2.23 b	1.79
Siderite	6.87	2.72 a	2.03
A. retroflexus	6.45	2.62 a	2.13
A. retroflexus + $FeSO_4$	5.99	2.43 ab	1.87
Significance	ns	*	ns
Biochar (g kg^{-1})			
0	4.86	1.83	0.98
50	7.46	3.23	2.97
Significance	***	***	***
Fert x Biochar	ns[1]	ns	ns

[1]ns, * and *** = effect not significant or significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. When a significant effect occurred, means in the same column followed by the same letter are not statistically different ($P < 0.05$, SNK Test).

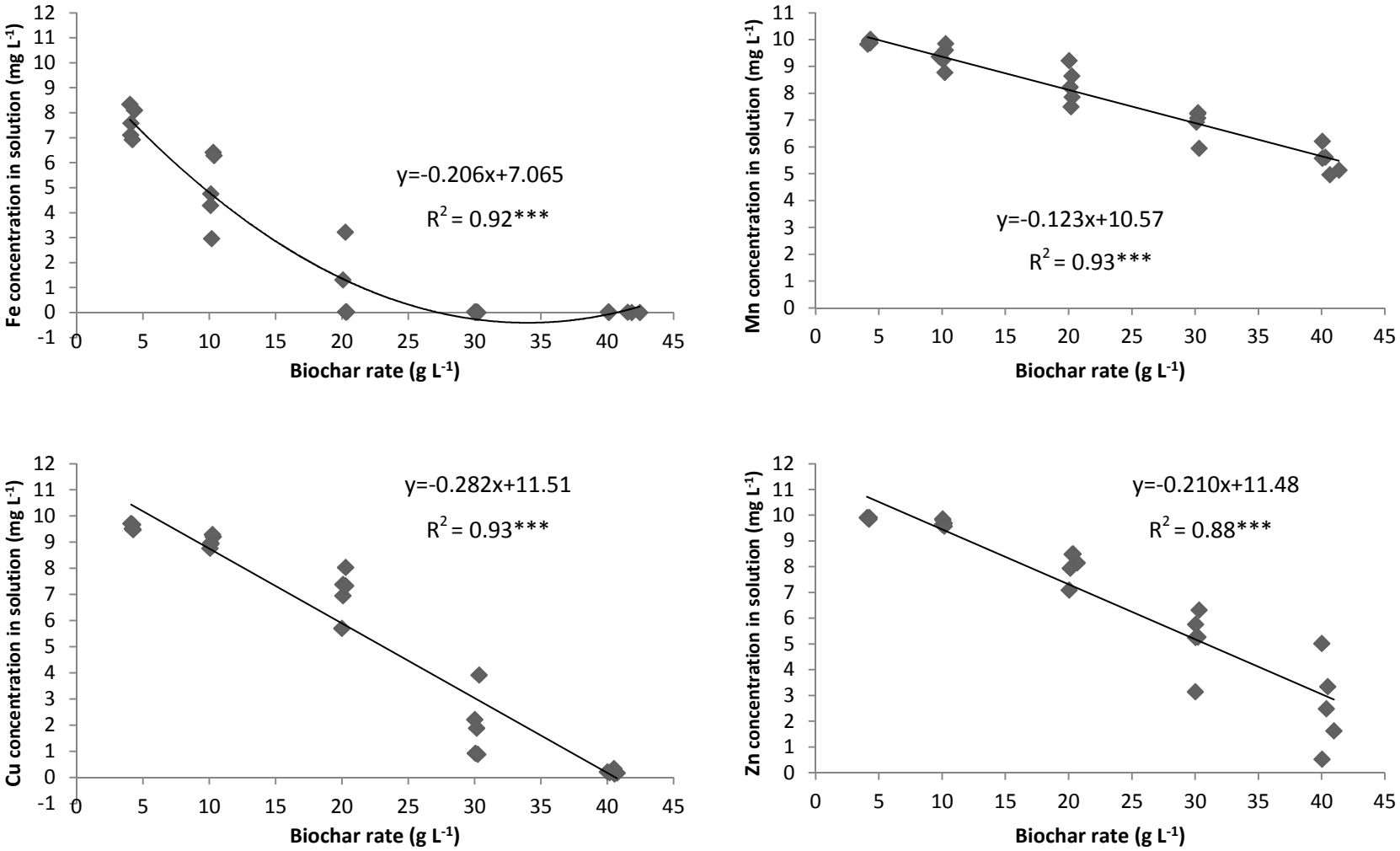

Figure 4.1. Effect of increasing rates of biochar on micronutrients retention potential

Fe, Mn, Cu and Zn concentration in deionized water (d-H_2O) was 0.022 ± 0.002, 0.016 ± 0.01, 0.0003 ± 0.0001 mg L^{-1} and < dl (avg. ± SE; n=5), respectively. Fe, Mn, Cu and Zn concentration in solution was 9.28 ± 0.036, 10.05 ± 0.0, 9.88 ± 0.0007 and 10.01 ± 0.0002 mg L^{-1} (avg. ± SE; n=5), respectively. ***: correlation between biochar rate and mineral concentration significant at P≤0.001.

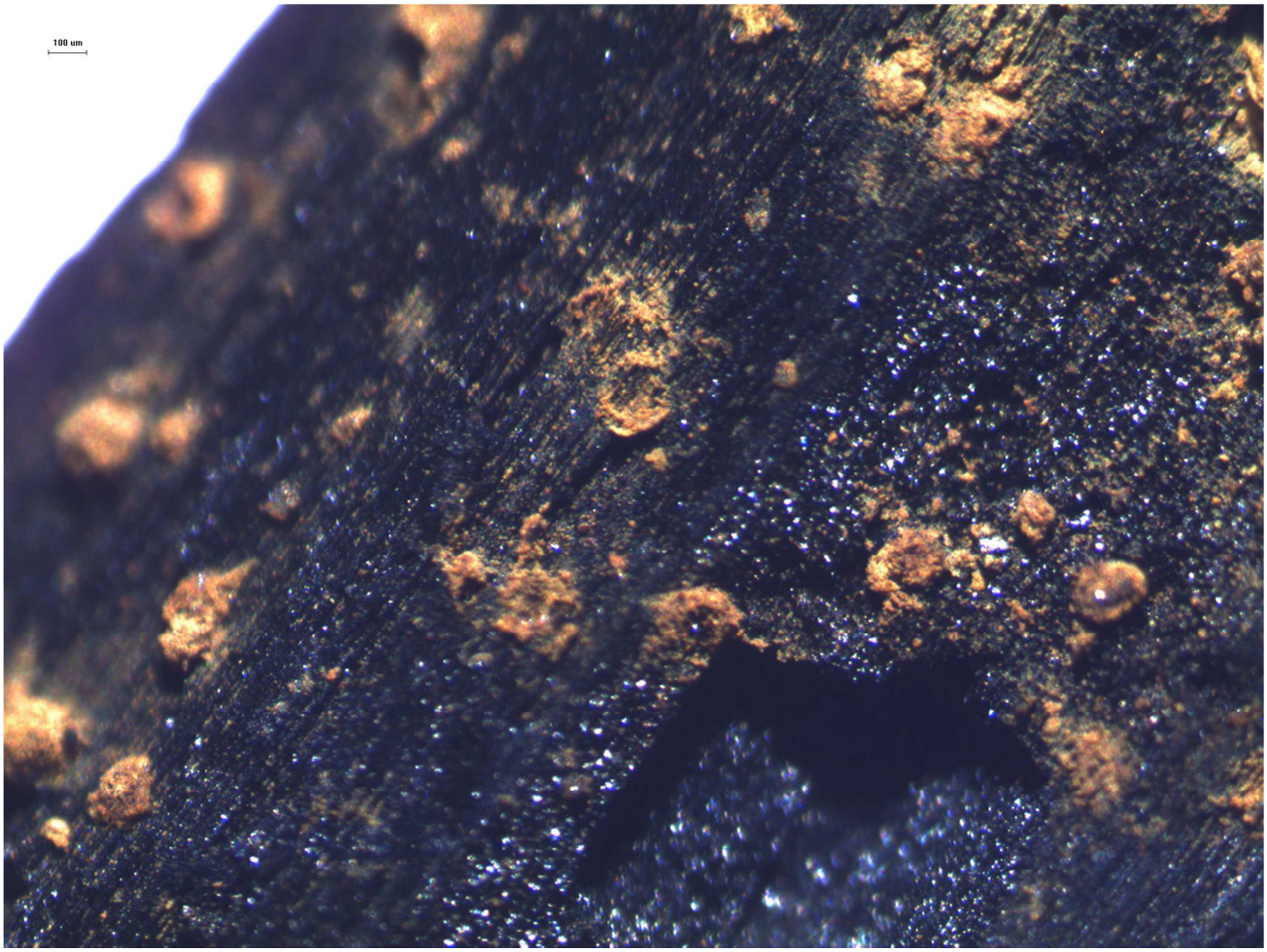

Figure 4.2. Magnification (3.2x) of a biochar fragment recovered after 1-week exposure to a source of Fe. Likely due to redox reactions, typical rusted spots are clearly evident. The biochar surface (top 5 nm) was then scanned by X-ray photoelectron spectroscopy (XPS). Magnification was obtained by an Olympus SXZ16 microscope coupled with an Olympus digital camera.

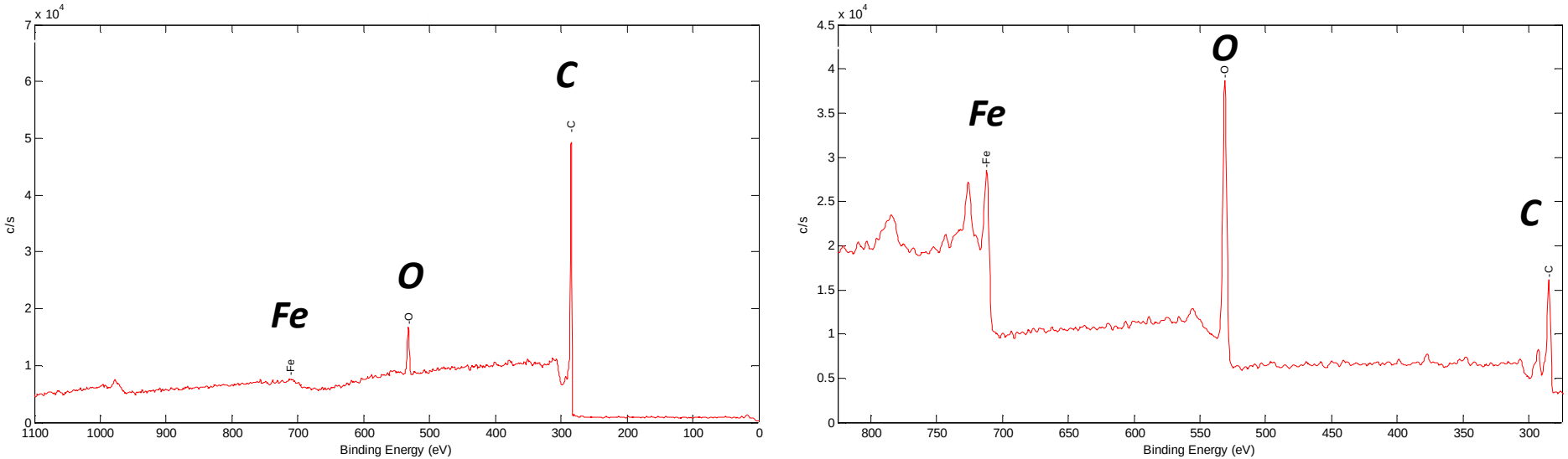

Figure 4.3. X-ray photoelectron spectroscopy (XPS) spectra of the relative surface atomic (%at) Fe, O and C composition of biochar fragments (top 5 nm). On the left, spectra of the biochar surface exposed 1-week to deionized water (d-H_2O) and (right) to a Fe source in solution. The latter shows a relatively higher concentration of Fe and O, suggesting that biochar interacted with the metal

CHAPTER 5

Soil leaching properties as affected by biochar and compost: a lysimeter experiment on nectarine trees

Abstract

The aims of this study were to assess the effect of soil-applied biochar, compost or their mixture on the leaching volume and chemical losses. To this end, a 2-year experiment on nectarine trees (Big Top/Adesoto 101) grown in lysimeters filled with 503 kg each of a sandy soil was carried out. Single 1-year old trees were planted in lysimeters and watered by microirrigation. In a complete randomized experimental design with 4 replicates the following soil-applied strategies were compared: a) unamended control (mineral source of nutrients); b) hardwood biochar (at a rate of 20 g fw kg^{-1} w w^{-1}); c) compost (at a rate of 76.8 g fw kg^{-1} w w^{-1}); d) hardwood biochar+compost (same rates of the previous two strategies). Amendments were applied at the beginning of the experiment. From April 2012 to March 2013, leachate was daily collected and monthly cumulated. Each month a subsample was analyzed for pH, electrical conductivity (EC), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), mineral nitrogen (N), as well as for macro, micronutrients and heavy metals concentration. Similarly, samples of rain and irrigation water were monthly collected and analyzed. The same procedure was adopted to collect the leachate in Sep-2013.

Compost increased leached volumes and soil water holding capacity (WHC) compared to unamended and biochar amended soils because of the ability of organic matter (OM) in retaining water, suggesting that its application may contribute to increase water use efficiency in croplands. Mixing compost with biochar reduced leaching volumes compared to compost alone while shifting up soil field capacity, proposing synergistic effects. Compost-treated soils dramatically increased DOC, TDN, mineral N and chemicals flushed down through leaching. A synergist effect between the two amendments was identified in the leaching of DOC, although the origin of the extra rate remains unknown. Ag, Be,

Cd, Sb, Ti and Tl were never detected in the leachate, while heavy metals (i.e. Ni, Pb, Cr and V) were detected in traces, although their concentration remained below the limits for drinking water. Independently of the strategy, the most leached elements were Ca, S and Na, which were also the most represented elements supplied to the soils by the irrigation water. Biochar sporadically reduced the leaching volumes compared to unamended soil and its addition increased the leaching of Hg, K, P, Mo, Se and Sn. However, unless for K and P, values were in the order of few tens of g ha^{-1} $year^{-1}$. We provided evidences of the leachate composition and estimated nutrient losses which may have agronomical and environmental implications.

Keywords: Nutrient leaching, Leachate, DOC, TDN, DON, biocompost

5.1 INTRODUCTION

Leaching in soil greatly affects nutrient cycling in agriculture (Brady and Weil, 2008) and contributes to deplete fertility of highly permeable soils (Steiner et al., 2008). Nutrient leaching occurs when mobile ions dissolved in soil solution move outside the rooting zone, making them unavailable for plant uptake (Major et al., 2009) and a potential hazard for groundwater use (Sunitha et al., 2012). Water percolation through the soil profile depends mainly on the soil infiltration capacity (hydraulic conductivity) which is associated with soil texture, minerals and organic matter (OM) content, water retention on the root zone and crop transpiration rate that are in turn related to the density and the ability of roots to absorb water. In addition, atmospheric precipitations (in terms of intensity, timing and amount), irrigation volumes, rate and chemistry of the elements in soil solution, timing and placement of synthetic and/or organic fertilizers significantly affect nutrient leaching patterns in croplands (van Es et al., 2002; Cahn et al., 1993). For instance, whether a nutrient in soil is under organic or inorganic form, its size and charge properties determine how it interacts with other particles in the soil matrix. In fact, positively charged ions or molecules can be retained by negatively charged clays and soil OM (Brady and Weil, 2008) particles. Similarly, negatively charged ions (e.g. Nitrate-N (NO_3^--N) can be retained by positively charged compounds.

Biochar is the carbon(C)-rich residue of biomass pyrolysis intentionally applied to crop lands with the purpose to sequester photosintetically fixed C, hence potentially mitigating climate changes (Woolf et al., 2010), as biochar in the soil system is thought to be stable even for thousands of years (Glaser et al., 2002). Due to its intrinsic properties that develop over time through surface oxidation and interaction with plant–soil–microbial components (Spokas et al., 2012; Downie et al., 2009), biochar has been proposed as a strategy to ameliorate soil properties and growth conditions (Spokas et al., 2012; Verheijen et al., 2010). Mechanisms that have been suggested to explain how biochar in soil might benefit plant growth and crop yield include alteration of soil chemistry (direct source of nutrients or improvement of nutrient efficiency), modification of the nutrient dynamics in soil and/or altering soil reactions by providing chemically active surfaces and shift of soil physical parameters that benefit root growth and/or nutrient, water retention

and uptake (Ippolito et al., 2012; Sohi et al., 2010; 2009; Lehmann and Joseph 2009). It has been proved that the application of charred biomasses represents an effective, long-term tool for reducing the adverse impact of mineral leaching on surface and groundwater quality (Ding et al., 2010; Laird et al., 2010; Steiner et al., 2008; Lehmann et al., 2003). Biochar in soil, in fact, may act as a sponge, soaking up different forms of OM as well as water and nutrients (Glaser et al., 2002) as reported by previous experiences which demonstrate the effectiveness of biochar in increasing soil WHC (Baronti et al., 2014) and reducing losses of many ions (at least in the short-term) through leaching including Ca, magnesium (Mg), phosphate (PO_3^-), ammonium-N (NH_4^+-N) and NO_3^--N (Kammann et al., 2014; Ventura et al., 2013; Laird et al., 2010; Lehmann et al., 2003). Higher nutrient retention after charcoal addition was observed by Glaser et al. (2002) who concluded that charcoal contributed to an increase in ions retention and to a decrease in the leaching of dissolved OM and organic nutrients in acidic tropical soils. The addition of biochar, in fact, slows transport of nutrients through the soil profile and therefore, keeps them available for uptake by plant roots for a longer period (Sun et al., 2015).

However, due to the complex nature and the heterogeneity of biochars, its predictive behavior in different soils and how to best optimize the potential useful characteristics of biochars are yet to be established.

Compost is defined as the stabilized organic amendment resulting from the biodegradation of a wide range of organic substrates (by-products) through the action of various microorganisms under aerobic conditions. Its use in fruit trees ecosystems is progressively gaining interest as a mean to enhance and restore soil OM (Diacono and Montemurro, 2009) and as a source of plant available nutrients, including N, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) as well as a range of essential trace elements (Smith and Collins, 2007; Haug, 1993). As a consequence, compost can be profitably adopted as a fertilizer in agriculture (Baldi et al., 2010; Caballero et al., 2009) and permits to recycle municipal solid and agri-food industry related wastes, offering environmental advantages and reduction of social costs. Although agronomical benefits have been confirmed (Baldi et al., 2010, Hargreaves et al., 2008), concerns about the use of

compost in agriculture have been raised since it may increase the availability of soil NO_3^--N that can be easily leached out from the soil profile. In addition, heavy metals such as lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn) as well as organic toxins (Giusquiani et al., 1995) can be added to soils upon low quality compost addition, thus potentially increasing soil and groundwater pollution through leaching.

However, despite considerable research has been conducted on the fate of nutrients in compost-amended agricultural soils (Johnson et al., 2004), there are still essential lacks of knowledge in this field since compost amendment includes a high variability of materials.

To date, most of the literature evaluated the effect of either soil-applied compost or biochar mainly on PO_3^-, NH_4^+-N and NO_3^--N ions (Ding et al., 2010) or heavy metals (Wang et al., 2008), while much less is known about their effects on a wide range of chemicals. Fate and estimate of dissolved organic C (DOC), total dissolved N (TDN) and nutrients fluxed out through leaching in biochar and compost-amended soils is important, as off-site movement has the potential to impact adjacent terrestrial or aquatic ecosystems (Jacinthe et al., 2004). However, literature reports results often obtained from short-lived experiments (<6 months), frequently carried out adopting the soil-column or the suction cup approach and in non-temperate soils under leaching conditions in which information are still insufficient, with effects expected to differ from tropical soils. Even less is known about leaching characteristics when biochar is mixed with organic amendments, such as compost.

This study was undertaken to assess the effect of soil-applied biochar or compost as well as their mixture on the volume and chemical properties of the leaching solution monthly drained from lysimeters in which a non-bearing nectarine tree was grown. Results were then used to estimate the losses of chemicals through leaching on a hectare basis.

5.2 MATERIALS AND METHODS

5.2.1 Experimental conditions

We performed a 2-year experiment (2012-13) outdoors at the experimental station of the University of Bologna (in Cadriano, Bologna, 44°54' N, 11°41' E, 36 m a.s.l.) on 1-year old nectarine trees (*Prunus persica* (L.), Batsch) of the cv. Big Top grafted on Adesoto (formerly Puebla de Soto 101 - *Prunus insititia* (L.), Bullace) grown in lysimeters of a volume of 0.496 m^3 ea. Lysimeters were 0.112 x 0.103 x 0.043 m (Le, L, H), internally lined with a plastic isolating film, provided with a 2-way outlet located at the bottom (Pic. 5.1 and 5.2) and filled with 503 kg ea. of a sandy soil (Tab. 5.1).

Picture 5.1. The lysimeters used in the experiment

Lysimeters were arranged in a single row, spaced 50 cm between each other and N-S oriented. At the end of March 2012, single trees were transplanted in the lysimeters, trained as slender spindle, covered (in summer) with a shade netting which allowed a max light intensity of 89.500 Lx (measured in summer at noon on a sunny day), watered daily (from May to September) by microirrigation (4

drippers per plant of 2 L h^{-1} ea.) to return the evapotranspiration (ETo) rate as estimated by a class A evaporation Pan and the specific crop coefficient (Kc) for nectarine, whereas weeds were manually removed.

Picture 5.2. Leached soil solution was collected in tanks at the bottom of the lysimeters

Climate of the area is classified as temperate sub-continental with cold winters, humid and warm summers. Throughout the experiment, irrigation volumes and meteorological data (daily precipitation, air temperature and relative humidity) were recorded by an automated weather station available at the experimental farm.

5.2.2 Experimental design and treatments

In a complete randomized experimental design with 4 replicates (single lysimeter) the following soil-applied amendment strategies were compared: a) unamended control (mineral source of nutrients); b) biochar (at a rate of 20 g fw kg^{-1} w w^{-1}) equal to 87.4 t fw ha^{-1} (considering a treated area of 1 ha, soil incorporation up to

0.35 m depth and a specific soil weight of 1.248 t m^{-3}); c) compost (at a rate of 76.8 g fw kg^{-1} w w^{-1}); d) biochar+compost (named biocompost from now on) (at the same rates of the previous two strategies). Amendments were applied once at the beginning of the experiment and carefully homogenised with the soil before filling the lysimeters. Unamended and biochar-amended soils received 41.7, 9.3 and 6.9 g pot^{-1} of N, P and K, respectively in the first season and 62.4, 12.0 and 22.9 g pot^{-1} of nitrogen (N), P and K, respectively. Urea (46% N), ammonium-nitrate (NH_4NO_3) (27% N) and complexed NPK (14-25-5 + SO_3 + microelements) commercial fertilizers were used as a source of nutrients. Mineral fertilizers were applied from growth resumption until the end of the vegetative season, at about 2 weeks intervals and supplied by fertigation. Compost-based amended soils received the same amount of tap water in coincidence with fertigation events.

Table 5.2 summarize physical-chemical biochar characteristics which was produced in a traditional charcoal kiln by a mixed feedstock of chipped hardwood (mostly from peach and grapevine), slowly pyrolized at approximately 550°C.

The compost used in the experiment was obtained by the biological decomposition of organic municipal wastes (85%) mixed with pruning material from urban ornamental trees and garden management (6.5%) and agro-industrial organic residues (8.5%), after a 3-month aerobic stabilization. Main physical and chemical characteristics of the compost are summarized in table 5.3.

5.2.3 Leachate recovering and sampling

From April 2012 to March 2013, when present, leachate was daily collected from the tanks located under the lysimeters and the volume was measured. Leached solution monthly recovered from the same lysimeter was cumulated and stored at 4°C. At the end of each month, a subsample of about 500 mL $lysimeter^{-1}$ was collected from the cumulated volume and stored at -20°C to await analysis.

The same procedure was then adopted for the leaching solution collected in September-2013, 17 months after the trial establishment.

5.2.4 Rain and irrigation water sampling

If rain events occurred, three samples per month were collected and similarly, three samples per month of irrigation water (tap water) were collected during the irrigation season. Unless differently specified, samples were then analyzed as described for the leachate.

5.2.5 Chemical Analyses

5.2.5.1 pH and electrical conductivity

The pH and electrical conductivity (EC) were measured under continuous stirring by a pH-meter (BasiC 20, Crison, Barcelona, Spain) and a conductimeter (CDM210 Conductivity Meter, Radiometer Analytical, Copenhagen, DK), respectively. Data of CE were adjusted to the temperature of 20° C through the conversion factors;

5.2.5.2 Dissolved organic C (DOC) and total dissolved N (TDN) concentration

DOC and TDN were determined by an elemental analyzer TOC-Vcpn-TNM1, (Shimadzu Corp., Kyoto, Japan). The analyzer injected 50 µL of a solution acidified with 2M hydrochloric acid (HCl), in order to eliminate inorganic C, into a combustion furnace held at 725 °C after which the CO_2 produced is detected by an infra-red gas analyzer and the mono-nitrogen oxides (NO_x) via a chemiluminescence detector. The instrumental detection limit (DL) for DOC and TDN was 50 µg L^{-1}.

5.2.5.3 Soluble NO_3^--N and NH_4^+-N concentration

NO_3^--N and NH_4^+-N concentration was determined by an autoanalyser (Auto Analyzer AA-3; Bran+Luebbe, Norderstadt, Germany). NH_4^+-N was measured colorimetrically by the salicylate method. Briefly, the liquid sample reacts with salicylate and dichloroisocyanuric acid (with nitroprusside as a catalyst) to produce a blue compound measured at 660 nm (ISO/DIS 11372), while NO_3^--N was first reduced to nitric oxide (NO_2) by hydrazine in alkaline solution with Cu catalyst, followed by the reaction with sulphanilamide and N-1-napthylethylenediamine

dihydrochloride (NEED) to produce a pink compound measured at 550 nm (ISO/DIS 13359).

5.2.5.4 Dissolved inorganic N (DIN) and dissolved organic N (DON)

DIN was calculated as the sum of the N mineral forms (NO_3^--N and NH_4^+-N) while DON represents the difference between TDN and DIN, as described by Christou et al., 2005.

5.2.5.5 Elemental concentration

Ag (Silver), Al (Aluminum), As (Arsenic), B (Boron), Ba (Barium), Be (Beryllium), Ca, Cd, Co (Cobalt), Cr (Chromium), Cu, Fe (Iron), Hg (Mercury), K, Li (Litium), Mg, Mn (Manganese), Mo (Molibdenum), Na (Sodium), Ni (Nickel), P, Pb, S, Sb (Antimony), Se (Selenium), Si (Silicon), Sn (Tin), Sr (Strontium), Ti (Titanium), Tl (Tallium), V (Vanadium) and Zn concentrations of liquid samples were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), sequential and simultaneous (Ametek Spectro Arcos EOP, Kleve, Germany).

5.3 STATISTICAL ANALYSES

Values of DOC, TDN, DON, DIN, NO_3^--N, NH_4^+-N and elemental concentration were referred to a weight of 4.368 t of soil which represents the soil volume of 1 ha considering 0.35 m of soil depth and a specific weight of 1.248 t m^{-3}.

Data were analyzed according to repeated measures analysis of variance (ANOVA) with 4 replicates using PROC MIXED (Littell et al., 1998) with a compound symmetry covariance structure. Effect of the amendments in September 2013 was analyzed as in a complete randomized block design with 4 replicates. Homogeneity of variance was checked using Levene's test before analysis. When analysis of variance showed a statistical effect, means were separated by using Tukey's HSD Test (at $P \leq 0.05$). Statistical analyses were performed by using SAS software (SAS Institute Inc., Cary, NC, USA).

5.4 RESULTS

5.4.1 Climate and irrigation conditions

Average temperatures fluctuated from 2.3°C in December 12 to 26.5°C in July 13 (Fig. 5.1), while atmospheric relative humidity ranged between 55.6 and 78.9 % (data not shown). The annual precipitation (April 12/March 13) was 854.8 mm (Fig. 5.1), with few days of snow recorded in December 12 and March 13. Unless in summer, when scarce precipitations were recorded (20.4 mm from June to August), rainy days were uniformly distributed throughout the year with 87 days of rain (>1.0 mm) and only in 15% of the events, precipitations were higher than 20 mm day^{-1}. The most intense precipitation was recorded on February 2nd, 2013 (37 mm in 24 h) whereas the wettest month was September 12 (135 mm). In September 13, total precipitations were 30.2 mm (air temperature was in average 20.4 °C) with 3 rainy (>1.0 mm) events (data not shown).

Irrigation was provided from April through September 12 with a total volume of 971 mm, mainly supplied from Jun through August (Fig. 5.1). In September 13, 58.6 mm were supplied trough irrigation.

5.4.2 Leached volumes

High volumes of leachate were collected in winter, with almost 90 mm recorded in February (Fig. 5.2). The lowest volumes were recovered in summer, particularly in August, whit intermediate values in spring and autumn (Fig. 5.2). No leachate was collected in October (Fig. 5.1). With the exception of September 12, January, February and March, treatments significantly affected the amount of solution monthly recovered (Fig. 5.2). The highest volumes were collected from soils amended with compost (either alone or mixed with biochar), without significant differences induced by biochar (Fig. 5.2). Leaching volumes were significantly increased by the incorporation of compost in 3 and 7 months compared to unamended and biochar-treated soils, respectively, while only in November a significant higher volume was recorded in biocompost in comparison with the control (Fig. 5. 2). Unless when treatments did not affect leaching, biochar alone always reduced significantly the leached volumes compared to biocompost and, in three months (May, June and December), also to untreated control (Fig. 5.2).

However, only soil amended with biochar significantly reduced the cumulative leached volume collected in 1 year (April 12/March13) compared with compost alone by 68.4 mm, while intermediate amounts were recorded for unamended control and biocompost (Tab. 5.5). In September 13, after 17 months from trial establishment, soils amended with biochar (alone or in combination with compost), significantly decreased the leached volumes compared with compost alone (Tab. 5.5). Nevertheless, amended soils were not significantly different from unamended control (Tab. 5.5).

5.4.3 pH and EC

The pH of the leachate was sporadically affected by the amendments (Tab. 5.6 and 5.8). Compared to other treatments, biochar significantly increased the pH of the leaching solution in March and September 2013 and, in the latter month, a similar result was induced by biocompost (Tab. 5.6 and 5.8). However, independently of the treatment, pH remained relatively constant and values ranged between 7.28 and 8.30, comparable with that of the irrigation water (7.59 ± 0.12), but higher than rainfall (6.42 ± 0.17).

Overall, the addition of compost (alone or in combination with biochar) significantly increased the EC of the leachate by 3-folds (Tab. 5.6), while similar values were observed between biochar and unamended control (Tab. 5.6 and 5.8). Compared to compost alone, the EC of the solution was increased by biocompost in May, February and March (Tab. 5.6). EC values were meaningfully higher in average by 3 times than irrigation and rainfall water.

5.4.4 Dissolved organic C and N forms

Unlike in August, when no differences were induced by treatments (Tab. 5.7), the addition of compost (either alone or mixed with biochar) significantly increased the overall amount of DOC and N forms collected during the first year of experiment compared to other treatments (Tab. 5.7), whereas values between soil-applied biochar and untreated soils were always similar (Tab. 5.7), even after 17 months of experiment (Tab. 5.8). When compost and biochar were mixed together, a synergism in the amount of leached DOC, TDN and DIN during winter (from

January to March) was observed (Tab. 5.7). The amount of NH_4^+-N in the leachate was increased in the soils fertilized with N mineral sources, but only in September 12 (Tab. 5.7).

The cumulative (April 12/March 13) amount of DOC, TDN and DON found in the leachate collected from soils amended with compost was significantly increased (Fig. 5.3). In particular, biocompost promoted the highest amount of leached TDN (Fig. 5.3), while biochar alone did not differ from the untreated control (Fig. 5.3). Compost increased the cumulative amount of NH_4^+-N compared to biochar (Fig. 5.3). Values of DOC, TDN and DON were increased in September 13 by the application of compost alone compared to other treatments (Tab. 5.8).

5.4.5 Chemicals in leaching solution

Ag, Be, Cd, Sb, Ti and Tl concentrations in the leachate were always below the instrumental DL (Tab. 5.9). In addition, As, Co, Cr, Hg, Pb, Se, Sn, and V were not detected in the leachate of September 13 (data not shown) and Al, As, Co, Hg, Pb, Sn and V were occasionally detected in traces (data not shown). Independently of the amendment, the most abundant chemicals leached in 1 year, as well as in September 13, were Ca (614 kg ha^{-1} $year^{-1}$), S (359 kg ha^{-1} $year^{-1}$), and Na (224 kg ha^{-1} $year^{-1}$) (Tab. 5.9), while Sn and Cr accounted for the lowest values, equal to less than 2 g ha^{-1} $year^{-1}$ (Tab. 5.9). Cumulative leached amount exceeding 0.8 kg ha^{-1} $year^{-1}$ were recorded for Mg, Si, K, Sr, B and P (Tab. 5.9), while values for the other elements were less than 0.4 kg ha^{-1} $year^{-1}$ (Tab. 5.9). Unlike for Ca, S and Zn, the addition of compost significantly increased (mostly without interaction with biochar) the leached cumulative amount of Al, B, Ba, Cr, Cu, Fe, K, Li, Mg, Mo, Na, Ni and Sr compared with biochar alone and untreated control (Tab. 5.9). A synergistic effect between the two amendments was observed for Co, K, Li, P, Pb, Se and V (Tab. 5.9). Biochar significantly increased the amount of Hg and Sn in the leaching solution (Tab. 5.9) and decreased, only when supplied alone, the amount of Co and Fe. When compared to the unamended soil, biochar promoted the leaching of K, Mo, P, Se and Sn, whereas statistically comparable results were recorded for the other elements (Tab. 5.9). Similar trends were also observed for the leachate collected in September 13 (data not shown).

5.5 DISCUSSION

5.5.1 Volume of the leaching solution

The incorporation of the amendments into the soil significantly affected soil leaching properties in terms of volume and chemical characteristics. However, treatments did not influence the leaching volume in September 12 and during winter (January, February and March), when highest values were recorded (>50 mm month^{-1}). When leached volumes monthly recovered were higher than this value, treatments did not induce differences. This response is likely due to frequent, time limited and intense atmospheric events (heavy rain and snow) concentrated in few days, associated with low temperatures and high relative humidity (UR%) with negligible transpiration rates that could not have reduced water percolation through the soil profile with these conditions. Due to these circumstances, the soil was often water-saturated, thereby the exceeding volumes were leached down without interference of the treatment.

In the other months, the highest volumes of leachate were constantly recorded from the soils treated with compost and biocompost. This seems related to the effect of compost on the soil WHC, which in turn affected the soil field capacity (FC). The latter is mainly controlled by pore size distribution in soil, since only pores less than 50 μm in diameter are able to retain water by capillary force. Many studies demonstrate the beneficial impact of the organic amendments (including compost) on the soil WHC and FC (Evanylo et al., 2008; Liu et al., 2007; Tejada et al., 2006; Carter et al., 2004). OM can take up and firmly retain water up to 20 times its own weight (Reicosky, 2005). Hudson (1994) showed an increase equal to 3.7% in the available soil WHC per increasing unit of soil OM.

In our conditions, the water retained in soil amended with compost lasted longer than in other treatments and, since the irrigation rate and timing were not different among treatments, the additional supply of water through irrigation (or from atmospheric precipitations) promoted higher leaching volumes. This means that the soil treated with compost was frequently at FC or above it as also confirmed by the soil water content (SWC), which resulted always increased in the compost-amended soil (data not shown), confirming that less irrigation volumes or extended intervals between two consecutive irrigations could have been adopted, with no

reduction of water availability for crop growth. Based on these observations, the incorporation of compost in croplands can be indicated as a valuable strategy to save water in agriculture.

Biochar significantly decreased the volume of the leachate compared to unamended soil in three months, although it was not enough to reduce the cumulated (1-year) volume. Similar volumes with the unamended soil were then recorded in September 13 (after 17 months from biochar incorporation). However, the addition of biochar reduced the leached volume by 37.6 mm $year^{-1}$, equal to near the 10% of the total volume leached by the untreated soil. Altering soil hydrology, including changes in infiltration and drainage rates, shift in the amount of water stored in soils, and shift in soil hydrophobicity, biochar has been suggested to provide a long-term modification in water cycling and ecosystem processes mediated by water, thereby changes on soil WHC upon biochar addition are expected (Sohi et al., 2010). Although results are sometimes conflicting (Abel et al., 2013; Verheijen et al. 2010), most of the studies on soil-biochar mixtures have shown an increase in soil WHC up to 30% (Lei and Zhang, 2013; Basso et al., 2013; Novak et al., 2012) with positive implications in plant response (Kookana et al., 2011; Atkinson et al., 2010; Sohi et al., 2010) and plant water use efficiency (Baronti et al., 2014; Downie et al., 2009). The beneficial influence of biochar on soil WHC has been mainly linked to the increased surface area and to its porous structure which can absorb and retain water (Verheijen et al., 2010; Downie et al., 2009). Nevertheless, a further explanation could be due to biochar induced changes in the distribution and connectivity of pores in the soil environment. In fact, a large percentage of the biochar pores are smaller than 2×10^{-3} μm (Tseng and Tseng, 2006), contributing to reduce the water mobility through the soil since water moves better in pores in the order of a few tens of micrometers in size (e.g. 30 μm) (Brady and Weil, 2008). Once incorporated, biochar can modify soil porosity, thereby alter percolation and flow patterns (Major et al., 2009).

Biocompost reduced the leached volume recorded in September 13 compared to compost alone confirming the trend observed during the first years of investigation, suggesting a synergism between the two amendments. Furthermore, the soil amended with biocompost showed frequently a higher SWC, even significantly

greater than compost-treated soil (data not shown), suggesting an additive effect of biochar with compost, while maintaining comparable or reduced leaching volumes. We propose that compost and biochar, acting through different mechanisms, led to an additive effect in the SWC and, as a result, the soil FC shifted up. This synergism suggests that mixing biochar and compost represents a more effective agronomical strategy to increase the SWC than applying the two amendments separately.

5.5.2 Chemical properties of the leachate

The pH of the leachate remained unchanged, with few increase induced by the presence of biochar possibly related due to the alkaline nature (pH 9.8) of the charred biomass used in this experiment. The EC of the leachate was increased by compost and biocompost as a response of the high concentration of easily-soluble salts supplied with compost and values showed a gradual decline over 4 consecutive months after its incorporation (April 12), when the highest measures were recorded. After the initial 4-month period, EC values fluctuated between 0.55 and 2.60 mS cm^{-1}. Independently of the treatment, the lowest EC values were recorded in winter, likely as a consequence of a reduced soil mineralization rate, a lack of inorganic fertilizer inputs (limited to the unamended and biochar treated soils) and a dilution effect induced by the highest leached volumes, as mentioned above. The addition of biochar to soils has been suggested to increase the EC of leachate, because of the loss of salts (e.g. Na and K) from the biochar-soil matrix (Lehmann et al., 2003; Novak et al., 2009). However, in our conditions we did not observe such effect suggesting that this parameter is soil-biochar dependent. A significant increase in the EC of the leachate from biocompost was recorded in 3 months, compared to compost alone, suggesting a positive interaction between the two amendments. Since the EC of the leachate has been indicated as a possible index to evaluate the risk of groundwater pollution by dissolved ions (Ding et al., 2010), the interaction between compost and biochar should be further investigated.

5.5.3 DOC, TDN and N forms

In our experiment, the amount of chemical losses was estimated considering the concentration and the leached volume monthly recovered. Then, data were

expressed on a hectare basis, thereby results are influenced either by the absolute concentration and the volume. Furthermore, it must be mentioned that calculations here reported considered a soil volume of 1 ha with 0.35 m depth as like the entire surface would have received the amendments distributed uniformly. However, in field conditions, the amended area is often limited to a 2-m width strip along the tree rows (e.g. in a peach orchard whit a frame of 5 m x 3 m, only $^{2}/_{5}$ of the orchard surface is amended), thus our values could be overestimated if compared with traditional agronomic techniques.

Nectarine trees were grown in the lysimeters, hence plants may have affected leaching properties through uptake and/or releasing of organic compounds (root turnover and exudates). However, because plants were non-bearing (1-year old) and since at the end of the experiment plant biomass organs were similar among treatments (data not shown), we could assume that the influence of the plants was of minor importance and uniformly distributed among treatments.

Since the beginning of the experiment, relatively high amounts of DOC were recovered in the leachate. DOC fluctuated throughout the investigation period without a regular trend, although values were correlated with leached volumes. This response could be partially related to the soil type, since in sandy soil, as it was in our experiment (88% sand), mineralization is generally higher than in clay soils (Bernal et al., 1999). It is well known that depletion of degradable portion of soil C-compounds occurs faster with warmer temperatures while it accumulates during winter (Marschner and Kalbitz, 2003). As a consequence, it could be reasonable to consider that higher values of DOC flushed down should be observed in summer. Furthermore, it is reported that DOC in the leachate is highest in summer due to the contribution of root exudates and microbial metabolites (Marschner and Kalbitz, 2003; Kalbitz et al., 2000). We observed an opposite trend, since despite a lower concentration, the highest leached volumes were recorded in winter which in turn led to higher loss of DOC in autumn and winter than in spring and summer seasons.

Compost, either with or without biochar, meaningfully increased the leached amount of DOC and TDN monthly recovered in the percolated solution and, to a less extent, also the N forms compared to the other treatments. This trend was then

reflected on the cumulative amount of leached DOC and TDN, which increased in average by 4 and 2-fold, respectively compared to non-compost amended soils. However, because volumes and concentration of DOC and TDN of the leachate were always significantly increased in soils that received compost (alone or with biochar), possible dilution effects cannot change the meaning of the results.

The effect induced by compost was expected as it contains DOC (as well as complex mixture of both N inorganic and organic nature) as solid components in a range from labile to resistant to decomposition (Kaplan et al., 1995). Considerable increase in soil DOC has been reported after application of composts and/or organic amendments (e.g. manure) with immediate effects attributed to the dissolved organic matter of composts (Gregorich et al., 1998; Gigliotti et al., 1997). An increase in the DOC flux in the leachate was expected from biochar enriched soil, since an initial mineralization of pyrolized biomasses immediately after their incorporation in soils may occur (Barnes et al., 2014; Major et al., 2009; Cheng et al., 2008; 2006). Furthermore, some evidences indicate that biochar might stimulate the rates of loss of non-biochar C in soils (Wardle et al., 2008; Pietikäinen et al., 2000;) proposing either possible priming effect by decomposition of labile soil C (glucose) (Hamer et al., 2004) or sorption by biochar of compounds (i.e. phenols) which inhibit microbial growth (Gundale and DeLuca, 2007) as possible mechanisms. In our experiment, the DOC flux from biochar treated soil did not maintain a specific trend with time, suggesting that the easily leachable biochar-C fraction was negligible, and while significant amount of C-containing compounds were provided with biochar, we did not observe peaks in the leached DOC flux, suggesting that the release of DOC from biochar amended soils depends on the interaction between biochar and soil type. This response cannot be explained by the slightly reduced leached volumes compared to the unamended soil, since its concentration in the recovered solution was always comparable. However, biochar is also capable of sorbing soil C (Barnes et al., 2014), hence we cannot exclude that part of the DOC recovered in the leachate was biochar-derived. On the other hand, it is also supposable that a biochar-C fraction followed different fates (e.g. CO_2 flux, microbial biomass stimulation).

Interestingly, we observed a synergic effect between the two amendments in the amount of leached DOC and TDN, although limited in winter. The origin of the extra rate of DOC remain unclear since it could derive from an higher mineralization of compost induced by the presence of biochar or from an higher mineralization of biochar induced by compost or from the native soil OM (priming effect). A combination, at different rates, of these sources, can also be conceivable. Results from this study indicate that the total C concentration of the biochar fragments recovered at the end of the experiment from the soil treated with biocompost was similar to that of the fragments from biochar alone amended soil (data not shown), suggesting that a similar degradation process at the expense of the biochar particles occurred. Furthermore, because both DOC and TDN fluxes were increased we assume that an additional mineralization of compost may have occurred, since soil was N-poor and unlikely N from biochar would have increased TDN in the leachate. The significantly reduced amount of leached DOC observed after 17 months from biocompost compared with compost alone can be related to the reduced leached volume recovered in September 3.

Most of the TDN recovered in the leachate was inorganic and mainly as a NO_3^--N, while the NH_4^+-N fraction was often negligible. As expected, compost increased the amount of the N-forms in the leachate and a synergism with biochar emerged on the cumulative amount of TDN lost through leaching, mainly ascribed to the amount of NO_3^--N compared to the other forms. The concentration of the mineral N in the leachate (mainly NO_3^--N) collected from compost amended soils, often exceeded 50 mg L^{-1} (data not shown), although only during the first 6 months following its distribution, indicating that a rapid release of N from the compost used in the experiment occurred. After 17 months, the concentration of mineral N forms in the leachate was not affected by treatments, indicating that the effect of compost as a source of N was depleted.

In contrast with others studies, we did not observe any effect induced by biochar on the losses of DIN trough leaching. Biochar from bamboo applied at 0.5 % (w w^{-1}) showed a reduced NH_4^+-N concentration (supplied as ammonium chloride (NH_4Cl) in the leachate since it was retained in soil for a longer period time, suggesting that biochar acted as a nutrient-retaining additive and, therefore, could be used to

increase the efficiency of N fertilizers (Ding et al., 2010). Hardwood derived biochar was found to effectively reduce NO_3^--N leaching losses when applied at rates of 5 and 10 g kg^{-1} using swine manure (5 g kg^{-1}) as a source of N (Laird et al., 2010). Similarly, Ventura et al. (2013), showed a significant reduction in the leaching of NO_3^--N in an apple orchard fertilized with mineral N source. Our findings show that the effect of biochar on nutrient leaching in soil may vary with biochar and nutrient.

5.5.4 Macro and microelements

Sb and heavy metals were either never detected (i.e. Ag, Be, Cd, Ti and Tl) or detected in traces (i.e. Al, As, Co, Hg, Pb, Sn and V) in the leachate, without a clear trend or a treatment-induced effect. Nevertheless, the concentration of such elements monthly measured in the leachate was below the limits suggested by the Maximum Contaminant Level Goal (MCLG) for drinking water (US EPA, 2015).

Independently of the strategy, the most leached elements (cumulative) were Ca, S and Na. The same elements were the most supplied to the soil by the irrigation water, accounting for 666, 245 and 177 kg ha^{-1} $year^{-1}$, respectively. Similarly, significant amounts of Mg (164 kg ha^{-1} $year^{-1}$), Si (40 kg ha^{-1} $year^{-1}$), K (73 kg ha^{-1} $year^{-1}$) and TDN (23 kg ha^{-1} $year^{-1}$) were supplied to the soil, considering the contribution from both irrigation and rainwater. According to the water quality and mineral concentration, such values should be considered in the definition of the fertilization management, since for some elements, the amount supplied with irrigation and rain water may fulfill the yearly plant requirements.

The main effect in the nutrient losses through leaching was induced as a consequence of the incorporation of compost into the soil, either with or without biochar, which significantly increased the amount of most of the elements recovered in the leachate compared to other treatments. This is a response of both the increased leached volumes and the concentration in the liquid flushed down from soils amended with compost, while less evident were differences between biochar and untreated control. In most cases the addition of compost to the soil increased the cumulated amount of chemicals from 2 to 5-fold with a peak of 15-fold in the case of Cr. The relatively high concentration of chemicals in the

leachate of compost-treated soils is the results of the mineralization of the compost-matrix, as it releases elements under soluble forms which can be either uptake by plants or easily leached. Furthermore, the increasing in DOC concentration in soil due to the application of organic amendments, such as composts, has been indicated to influence movement of chemicals (e.g. nutrients and heavy metals) in soil (Wright et al., 2005), thus increasing the risk of leaching of metals and nutrients (Ashworth and Alloway, 2004), since as ions complexed with dissolved organic matter can readily move through soil (Kaschl et al., 2002).

The cumulated amount of Hg, K, Mo, P, Se and Sn in the leachate was increased by biochar compared with control, representing the soluble chemicals most released by biochar likely due of the biochar nature. Dissolution first and consequent leaching of soluble salts as well as organic compounds of the biochar are described among the first reactions occurring once biochar is incorporated into the soil, especially in irrigated soils or if a rain event occurs (Shinogi et al. 2003; Major et al. 2009). Lehmann et al. (2003) reported a much abundant content other than K, also of Ca and Mg in the leachate from biochar while Novak et al. (2009) reported an increase of K and Na concentration but a decrease of Ca, P, Mn, and Zn in the leachate from biochar indicating that the leachate composition is biochar-type dependent. Due to the dissolution of soluble salts from biochar, an increase in the pH and EC of the leachate was expected as also indicated by Joseph et al. (2010) which did not happen in our conditions. A synergic effect between biochar and compost led to an increase in the leached amount of K, Li, Se and Sn, while biochar alone reduced the losses of Co and Fe, although the latter differences are of few grams per hectare per year.

5.6 CONCLUSIONS

Our results suggest that the amendment with compost may significantly contribute to increase water use efficiency (either reducing irrigation volumes or delaying the interval between two consecutive irrigation events), while assuring adequate SWC to plants. Mixing compost with biochar seems even more effective since soil FC was shifted up, suggesting synergic effects induced by the combination of the two amendments. However, compost-based amended soils increased losses of DOC, TDN and mineral N forms through leaching with an additive effect between biochar and compost measured for DOC, although its origin remain unknown. This may have short-term adverse ecological implications (faster C losses in the environment), even though in the long term the potential to sequester C in soil by biocompost could even result enhanced compared to the mere addition of the amendments. However, measure of the C losses via leaching provided with this study may contribute to estimate the C sequestration potential of such strategies.

Nevertheless, in field conditions, we recommend to reduce the application rates of compost compared to this study or to split the yearly amount in 2-3 applications, starting few weeks earlier in relation to the highest plant N requirements in order to avoid excessive N losses. On the other hand, since no heavy detrimental effects were induced by biochar, application rates higher 20 g kg^{-1} can be supposed.

Irrigation water supplied significant amount of minerals in particular Ca, S and Na which reflected the order of the most abundant elements lost through leaching (666, 245 and 177 kg ha^{-1} $year^{-1}$, respectively). Significant amounts of Mg (164 kg ha^{-1} $year^{-1}$), Si (40 kg ha^{-1} $year^{-1}$), K (73 kg ha^{-1} $year^{-1}$) and TDN (23 kg ha^{-1} $year^{-1}$) were supplied to the soil considering the contribution from irrigation and rainwater. For some elements, the amount supplied with irrigation and rain water fulfills the yearly plant requirements.

Despite relatively high amount of minerals were lost through leaching when soil was amended with compost, we proved that biochar and compost of high quality and even their mixture, can be adopted as a sustainable agronomical strategy in terms of potential sources of heavy metals implied in groundwater pollution.

Biochar alone increased leaching of Hg, K, P, Mo, Se and Sn but, unless for K and P, values were in the order of few tens of g ha^{-1} $year^{-1}$.

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Table 5.1. Soil physical and chemical characteristics

Parameter	**Method**[1]	**Unit**	**Value**
Sand (2-0.05 mm)	Bouyoucos	g kg^{-1}	880
Silt (0.05-0.002 mm)	Bouyoucos	g kg^{-1}	90
Clay (<0.002 mm)	Bouyoucos	g kg^{-1}	30
Organic Matter	Walkley-Black	g kg^{-1}	5.5
Total C		g kg^{-1}	3.19
C/N ratio			7.78
pH (in water)			8.07
Total carbonate ($CaCO_3$)	De Astis	g kg^{-1}	190
Active lime ($CaCO_3$)	Drouineau	g kg^{-1}	1.1
Cation Exchange Capacity (CEC)	Barium Chloride	meq 100 g^{-1}	10.65
S.A.R. index			0.26
Chlorotic power index			71
Electrical conductivity		mS cm^{-1}	0.164
Total N	Kjeldhal	g kg^{-1}	0.41
Chloride water soluble		mg kg^{-1}	14
P exchangeable[2]	Olsen	mg kg^{-1}	26
P_2O_5 exchangeable[2]		mg kg^{-1}	60
K exchangeable[3]	Barium Chloride	mg kg^{-1}	87
K_2O exchangeable[3]		mg kg^{-1}	104
K water soluble		mg kg^{-1}	7.4
Ca exchangeable[3]	Barium Chloride	mg kg^{-1}	1914
Ca water soluble		mg kg^{-1}	71.9
Mg exchangeable[3]	Barium Chloride	mg kg^{-1}	79
Ma water soluble		mg kg^{-1}	4.8
Na exchangeable[3]	Barium Chloride	mg kg^{-1}	53
Na water soluble		mg kg^{-1}	8.3
Fe exchangeable[3]	DTPA	mg kg^{-1}	12.4
Mn exchangeable[3]	DTPA	mg kg^{-1}	6.2
Cu exchangeable[3]	DTPA	mg kg^{-1}	1.49
Zn exchangeable[3]	DTPA	mg kg^{-1}	0.76
B exchangeable[2]	hot water	mg kg^{-1}	0.32

[1]Analisys were performed according to National Official Methods (D.M. 13/09/1999 G.U. N, 248 of 21/10/1999).

[2]Determined spectrophotometrically

[3]Determined by AAS (Atomic Absorption Spectrophotometry)

Table 5.2. Biochar physical and chemical characteristics

Parameter	Unit	Value
Physical properties		
Moisture	%[1]	13.8
Bulk density	g cm^{-3}	0.43±0.04
Hydrophobicity		Slightly hydrophobic
Total porosity	mm^3 g^{-1}	2722
Transmission pores	mm^3 g^{-1}	318
Storage pores	mm^3 g^{-1}	1997
Residuals pores	mm^3 g^{-1}	406
Max water absorption	g g^{-1} of d.m.	4.53
Skeletal density (SD)[2]	g cm^{-3}	1.86±0.04
Envelope density (ED)[3]	g cm^{-3}	0.2459±0.0056
Porosity (ED/SD)	%	0.863±0.00574
Surface area[1] (BET Brunauer–Emmett–Teller method)	m^2 g^{-1}	410±6
Particle size distribution[1]	mm g^{-1}	
50-20	%	4.45
20-10	%	12.1
10-8	%	13.1
8-4	%	10.36
4-2	%	19.85
2-1	%	24.2
<1	%	15.94
Chemical properties		
pH	-	9.8
CEC	cmolc kg^{-1}	101
Carbon[1] (C)	g kg^{-1}	778.0
Total nitrogen (N)	g kg^{-1}	9.1
C/N	-	85.49
Aluminum (Al)	mg kg^{-1}	268
Arsenic (As)	mg kg^{-1}	0.005
Beryllium (Be)	mg kg^{-1}	0.001
Cadmium (Cd)	mg kg^{-1}	0.001
Calcium (Ca)	g kg^{-1}	25.0
Chrome (Cr)	mg kg^{-1}	0.002
Cobalt (Co)	mg kg^{-1}	0.002
Copper (Cu)	mg kg^{-1}	97
Iron (Fe)	mg kg^{-1}	333
Magnesium (Mg)	g kg^{-1}	28.7
Manganese (Mn)	mg kg^{-1}	84
Molybdenum (Mo)	mg kg^{-1}	2
Phosphorus (P)	g kg^{-1}	23.3
Potassium (K)	g kg^{-1}	13.9
Sodium (Na)	g kg^{-1}	11.9
Sulphur (S)	mg kg^{-1}	481
Zinc (Zn)	mg kg^{-1}	104

[1]data obtained from Baronti et al. (2014) (with permission). [2]The skeletal density is the sample mass divided by sample volume occupied by a solid sample, including any pores not accessible to the helium gas. [3]The envelope density is defined as the sample mass divided by the total sample volume that is measured if an "envelope" would be placed around each individual particle.

Table 5.3. Main physical and chemical compost parameters

Parameter	**Unit**	**Value**
Humidity	%	47.9
pH (in water)		7.5
Specific conductivity	dS cm^{-1}	3.52
Salinity	meq 100 g^{-1}	84.5
Plastic materials < 5 mm	% d.w.	<0.01
Plastic materials > 5 mm	% d.w.	<0.01
Other inerts < 5 mm	% d.w.	<0.01
Other inerts > 5 mm	% d.w.	0.33
Salmonella	[1]MPN g^{-1}	none
E. coli	[2]CFU g^{-1}	<25
Organic matter	g kg^{-1} (d.w.)	543.1
Organic Carbon (C)	g kg^{-1}(d.w.)	386
Humic and Fulvic C	g kg^{-1}(d.w.)	141
Total nitrogen (N)	g kg^{-1}(d.w.)	22.7
Organic N	% of total N	87.2
C/N		17.0
Chrome hexavalent (Cr)	mg kg^{-1}	<0.5
Cadmium (Cd)	mg kg^{-1}	<0.5
Sodium (Na)	mg kg^{-1}	3385.3
Lead (Pb)	mg kg^{-1}	31.1
Copper (Cu)	mg kg^{-1}	87.1
Zinc (Zn)	mg kg^{-1}	189.8
Mercury (Hg)	mg kg^{-1}	<0.5
Nickel (Ni)	mg kg^{-1}	15

[1]most probable number
[2]colony-forming unit
Source: Nuova Geovis, Bologna, Italy, (2012) – Analyses report N. 11.4235

Table 5.4. Chemical elemental concentration (µg L^{-1}) of the rain and irrigation water (mean ±SE)

Rain	Al	B	Ba	Ca	Cu	K	Li	Mg	Mn	Na	S	Si	Sr	Zn
Mean	24.7	5.8	4.0	3144.6	21.4	1036.2	3.2	432.4	2.3	4084.0	873.8	77.2	16.0	17.0
± SE	18.1	4.0	1.4	1305.9	7.4	109.0	0.3	155.7	0.8	1415.3	184.7	25.3	6.8	2.7

Ag, As, Be, Cd, Co, Cr, Fe, Hg, Mo, Ni, P, Pb, Sb, Se, Sn, Ti, Tl, and V concentration in the rainwater was below the instrumental detection limit (DL)
1.80 mg C (DOC) L^{-1} ± 0.19; 0.87 mg N (TDN) L^{-1} ± 0.19; 1.083 mg NO_3^--N L^{-1} ± 0.193 and 1.037 mg NH_4^+-N L^{-1} ± 0.176;
EC and pH (mean ±SE) was 27.7 ± 3.34 µS cm^{-1} and 6.42 ± 0.17, respectively;

Irrigation	B	Ba	Ca	Cu	Fe	K	Li	Mg	Mn	Na	Ni	P	Pb	S	Se	Si	Sr	Zn
Mean	120.3	68.8	79000	3.3	6.8	7600	21.4	18600	2.6	21500	8.3	25	2.8	29000	24.1	4600	642.9	18.8
± SE	4.1	14.7	12800	0.7	2.3	3000	1.9	3200	0.6	2100	5.2	5.3	2.7	3100	3.2	1900	257.9	5.8

Ag, Al, As, Be, Cd, Co, Cr, Hg, Mo, Sb, Sn, Ti, Tl, and V concentration in the rainwater was below the instrumental detection limit (DL).
4.77 mg C (DOC) L^{-1} ± 2.44; 2.67 mg N (TDN) L^{-1} ± 0.54; 1.037 mg NO_3^--N L^{-1} ± 0.262 and 0.160 mg NH_4^+-N L^{-1} ± 0111.
EC and pH (mean ±SE) was 482.7 ± 6.06 µS cm^{-1} and 7.59 ± 0.12, respectively.

Table 5.5. Effect of soil-applied Biochar and Compost on the cumulative leached volume recorded in 1 year (April 12 - March 13) and in September 13

Treatment	**Cumulative leached volume**	**Leached volume**
	mm $lysimiter^{-1}$ (April 12/March 13)	mm $lysimiter^{-1}$ (Sep-13)
Control	392.6ab	13.4ab
Biochar	355.2b	10.7b
Compost	421.4a	22.3a
Biocompost	406.3ab	7.4b
Significance	*	*

*: effect of the treatment significant at P ≤ 0.05. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test)

Table 5.6. Effect of soil-applied Biochar and Compost on electrical conductivity (EC) (mS cm^{-1}) and pH of the leachate throughout the experiment

Treatment	**Apr-12**		**May-12**		**Jun-12**		**Jul-12**		**Aug-12**		**Sep-12**		**Nov-12**		**Dic-12**		**Jan-13**		**Feb-13**		**Mar-13**	
	pH	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**	**pH**	**EC**
Control	7.49	0.82b	7.45	0.84c	7.67	0.81b	7.96	0.80b	7.82	1.44	7.73	2.21	7.94	2.28	7.69	1.93b	7.84	0.87b	8.04	0.40c	7.86b	0.38c
Biochar	7.28	0.91b	7.77	0.81c	7.38	0.79b	8.17	0.78b	7.65	2.12	7.78	2.26	7.90	2.15	7.80	1.67b	7.80	0.94b	7.98	0.47c	8.01a	0.40c
Compost	7.41	3.41a	7.79	2.07b	7.85	1.43a	8.06	1.06a	7.97	1.39	7.71	2.14	7.97	2.35	7.61	2.31a	7.61	1.10a	7.93	0.62b	7.85b	0.55b
Biocompost	7.61	3.73a	7.80	2.37a	7.80	1.60a	8.13	1.22a	7.78	1.77	7.76	2.56	8.11	2.56	7.63	2.60a	7.63	1.53a	7.97	0.78a	7.90b	0.64a
Significance	ns	***	ns	***	ns	**	ns	**	ns	ns	ns	ns	ns	ns	ns	**	ns	***	ns	***	*	***

In October 2012 no leachate was collected due to insufficient rainfalls.

ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test).

Table 5.7. Effect of soil-applied Biochar and Compost on the monthly amount of dissolved organic C (DOC), total dissolved N (TDN), total dissolved inorganic N (DIN), total dissolved organic N (DON), Nitrate-N (NO_3^--N) and Ammonium-N (NH_4^+-N) as measured in the leachate

Treatment	**Apr-12**	**May-12**	**Jun-12**	**Jul-12**	**Aug-12**	**Sep-12**	**Nov-12**	**Dec-12**	**Jan-13**	**Feb-13**	**Mar-13**
						DOC (kg C ha^{-1})					
Control	2.77b	4.82b	1.05b	1.14b	0.18	8.49b	2.13b	4.50b	11.4c	6.35c	3.64c
Biochar	1.65b	3.08b	0.81b	0.50b	0.05	8.44b	1.79b	3.15b	11.0c	6.12c	3.71c
Compost	29.7a	46.5a	9.78a	14.8a	0.83	35.2a	10.1a	22.5a	42.5b	32.5b	17.9b
Biocompost	40.9a	51.8a	9.39a	8.73a	0.60	39.6a	10.6a	27.0a	58.8a	91.1a	21.3a
Significance	***	***	*	*	ns	***	***	***	***	**	***
						TDN (kg N ha^{-1})					
Control	7.27b	30.7ab	1.31	0.29b	0.42	51.2	0.31b	0.50b	0.73b	0.55b	0.32c
Biochar	7.34b	17.3b	0.11	0.12b	0.33	39.7	0.19b	0.52b	0.78b	0.51b	0.42c
Compost	41.6a	49.6a	3.96	3.79a	0.99	21.1	1.21a	2.68a	4.50a	4.15b	2.05b
Biocompost	45.2a	51.3a	2.71	4.22a	0.85	37.1	1.45a	3.79a	6.64a	13.6a	2.95a
Significance	***	*	ns	*	ns	ns	**	***	***	***	***
						DIN (kg N ha^{-1})					
Control	7.00b	30.1ab	0.39	0.26	0.42	49.4	0.19ab	0.40	0.56b	0.05b	0.05b
Biochar	7.21b	16.6b	0.06	0.09	0.25	38.1	0.13b	0.44	0.58b	0.05b	0.05b
Compost	40.9a	48.2ab	0.72	3.22	0.36	18.4	0.43ab	0.99	2.53ab	0.92b	0.11ab
Biocompost	41.7a	50.6a	0.79	2.32	0.35	34.2	0.66a	1.63	508a	3.13a	0.35a
Significance	***	*	ns	ns	ns	ns	*	ns	*	***	*
						DON (kg N ha^{-1})					
Control	0.27b	0.63	1.00	0.02	0.14	1.82	0.13b	0.09b	0.17	0.50b	0.28b
Biochar	0.13b	0.68	0.08	0.18	0.06	1.61	0.05b	0.09b	0.20	0.46b	0.37b
Compost	1.10a	1.43	1.38	0.65	0.09	2.69	0.78a	1.69a	1.96	3.17a	1.93a
Biocompost	3.51a	0.68	0.80	1.86	0.50	2.94	0.79a	2.16a	1.56	10.5a	2.60a
Significance	*	ns	ns	ns	ns	ns	***	**	ns	**	***

continues...

Treatment	Apr-12	May-12	Jun-12	Jul-12	Aug-12	Sep-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13
						NO_3^--N (kg N ha^{-1})					
Control	5.66b	27.3ab	0.28ab	0.19	0.35	46.9	0.08	0.16	0.08b	0.01b	0.01b
Biochar	6.09b	14.6b	0.003b	0.40	0.25	35.9	0.04	0.23	0.09b	0.02b	0.01b
Compost	38.9a	45.3a	0.62ab	2.56	0.27	17.7	0.16	0.47	1.51ab	0.82a	0.05ab
Biocompost	40.1a	43.7a	1.58a	1.93	0.33	33.4	0.39	0.72	5.19a	3.01a	0.28a
Significance	***	*	*	ns	ns	ns	ns	ns	*	***	*
						NH_4^+-N (kg N ha^{-1})					
Control	1.34	2.78	0.02	0.07	0.01	2.52a	0.10b	0.24b	0.48b	0.04b	0.04bc
Biochar	1.12	2.01	0.06	0.06	0.01	2.24a	0.09b	0.21b	0.48b	0.03b	0.03c
Compost	1.59	2.90	0.096	0.66	0.10	0.71b	0.27a	0.52a	1.01a	0.10a	0.06ab
Biocompost	1.58	2.76	0.072	0.39	0.03	0.84b	0.26a	0.54a	1.06a	0.12a	0.07a
Significance	ns	ns	ns	ns	ns	***	***	***	***	***	*

In October 2012 no leached was collected due to insufficient rainfalls; dl: instrument detection limit.

DIN was calculated as the sum of the mineral N forms (NO_3^--N and NH_4^+-N) whereas DON was calculated as the difference between TDN and DIN.

ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test).

Table 5.8. Effect of soil-applied Biochar and Compost on the pH, EC, DOC (Dissolved Organic C), TDN (Total Dissolved N), DIN (Dissolved Inorganic N), DON (Dissolved Organic N), Nitrate-N (NO_3^--N) and Ammonium-N (NH_4^+-N) of the soil leached solution after 17 months since amendment incorporation (September 13)

Treatment	**pH**	**EC** mS cm^{-1}	**DOC** kg ha^{-1}	**TDN** kg ha^{-1}	**DIN** kg ha^{-1}	**DON** kg ha^{-1}	**NO_3^--N** g ha^{-1}	**NH_4^+-N** g ha^{-1}
Control	7.92b	1.56	2.12b	0.21b	0.014	0.20b	7.51	6.26
Biochar	8.08a	1.70	1.32b	0.13b	0.009	0.12b	7.20	2.01
Compost	7.94b	1.43	7.06a	0.60a	0.013	0.59a	9.53	3.78
Biocompost	8.30a	1.23	2.94b	0.18b	0.008	0.17b	7.49	0.74
Significance	***	ns	**	*	ns	**	ns	ns

Tap water (mean ±SE) = pH 7.29 ± 0.15; EC 846.7 ± 26.1 µS cm^{-1}; NO_3^--N 1.909 ± 0.45; NH_4^+-N <dl.
Rain water (mean ±SE) = pH 7.48 ± 0.21; EC 29.9 ± 4.11 µS cm^{-1}; NO_3^--N 0.273 ± 0.04 mg L^{-1}; NH_4^+-N 0.122 ± 0.09 mg L^{-1}.
ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test).

Table 5.9. Effect of soil-applied Biochar and Compost on the cumulative amount of chemicals leached in 1 year (April 12-March 13)

TRT	**Al**	**As**	**B**	**Ba**	**Ca**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**K**	**Li**	**Mg**	**Mn**	**Mo**	**Na**	**Ni**	**P**	**Pb**	**S**	**Se**	**Si**	**Sn**	**Sr**	**V**	**Zn**
	g ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	g ha^{-1}	g ha^{-1}	g ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	kg ha^{-1}	g ha^{-1}	g ha^{-1}
Control	4.86b	2.24ab	0.386b	239.2b	637.1	7.23b	0.06b	86.8b	19.7b	2.85c	15.7d	59.2c	78.9b	125ab	0.01c	138.5b	13.6b	0.276c	51.3b	378.0	18.5c	21.4ab	0.002b	2.958b	8.39b	62.0
Biochar	8.64b	1.68b	0.421b	239.3b	539.1	6.29c	0.07b	66.0b	7.02c	31.8a	43.6c	60.2c	79.1b	42.9b	4.90b	130.9b	10.7b	0.786b	44.8b	353.2	32.9b	18.6b	2.96a	2.733b	8.57b	52.3
Compost	47.4a	8.40a	1.191a	540.7a	658.9	7.96ab	1.17a	146.1a	217.3a	4.81c	143.6b	127.9b	112.4a	647a	19.2a	265.4a	47.0a	1.302ab	97.2ab	331.4	44.0b	38.1a	0.10b	3.650a	19.5ab	66.3
Biocomp	36.1a	8.04a	1.264a	571.2a	623.8	8.04a	0.90a	149.6a	313.9a	13.7b	259.8a	172.0a	131.6a	565a	34.6a	374.6a	39.1a	2.304a	135.5a	362.0	60.2a	26.4ab	2.65a	3.670a	29.2a	73.2
Sign.	*	*	***	***	ns	***	**	***	**	***	***	***	***	*	***	***	***	**	**	ns	***	*	**	**	**	ns

Ag, Be, Cd, Sb, Ti and Tl concentration in the leachate was always below the instrumental detection limit (DL).
ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test).

Table 5.10. Cumulative amount of DOC (Dissolved Organic Carbon), TDN (Total Dissolved Nitrogen) and minerals supplied by irrigation and rainfall water (kg ha^{-1} $year^{-1}$)

	DOC	TDN	Al	B	Ba	Ca	Cu	Fe	K	Li	Mg	Mn	Na	P	S	Se	Si	Sr	Zn
Irrigation	39.6	22.5	-	1.01	0.58	666.3	0.03	0.06	64.1	0.18	159.9	0.02	177.1	0.21	244.6	0.20	38.8	5.14	0.16
Rain	15.4	0.74	0.21	0.05	0.03	26.8	0.18	-	8.85	0.03	3.7	0.02	34.9	-	7.5	-	0.66	0.14	0.14

Ag, As, Be, Cd, Co, Cr, Fe, Hg, Mo, Ni, P, Pb, Sb, Se, Sn, Ti, Tl, and V concentrations in the irrigation water and Ag, Al, As, Be, Cd, Co, Cr, Hg, Mo, Sb, Sn, Ti, Tl, and V concentrations in the rainwater were below the instrumental detection limit.

Detection Limit ($\mu g\ L^{-1}$) of the ICP-OES unit (Avg ± SE) throughout the experiment (April 12-March 13)

Element	Ag	Al	As	B	Ba	Be	Ca	Cd	Co	Cr	Cu	Fe	Hg	K	Li	Mg
Value	6.2±5.4	8.4±5.3	5.6±1.7	3.0±0.8	11.6±10.9	1.8±1.6	6.7±3.0	1.9±1.1	2.2±1.5	2.6±1.4	3.2±1.9	5.2±3.0	3.4±2.6	2.5±1.1	3.1±3.0	7.7±2.1
Element	Mn	Mo	Na	Ni	P	Pb	S	Sb	Se	Si	Sn	Sr	Ti	Tl	V	Zn
Value	1.7±1.6	4.1±1.2	3±1.2	3.1±2.0	88.0±84.5	7.0±2.7	14.2±3.9	6.2±1	2.8±0.8	24.0±16.7	19.1±12.6	101.6±96.6	1.4±0.9	9.3±4.3	2.1±1.0	1.3±0.9

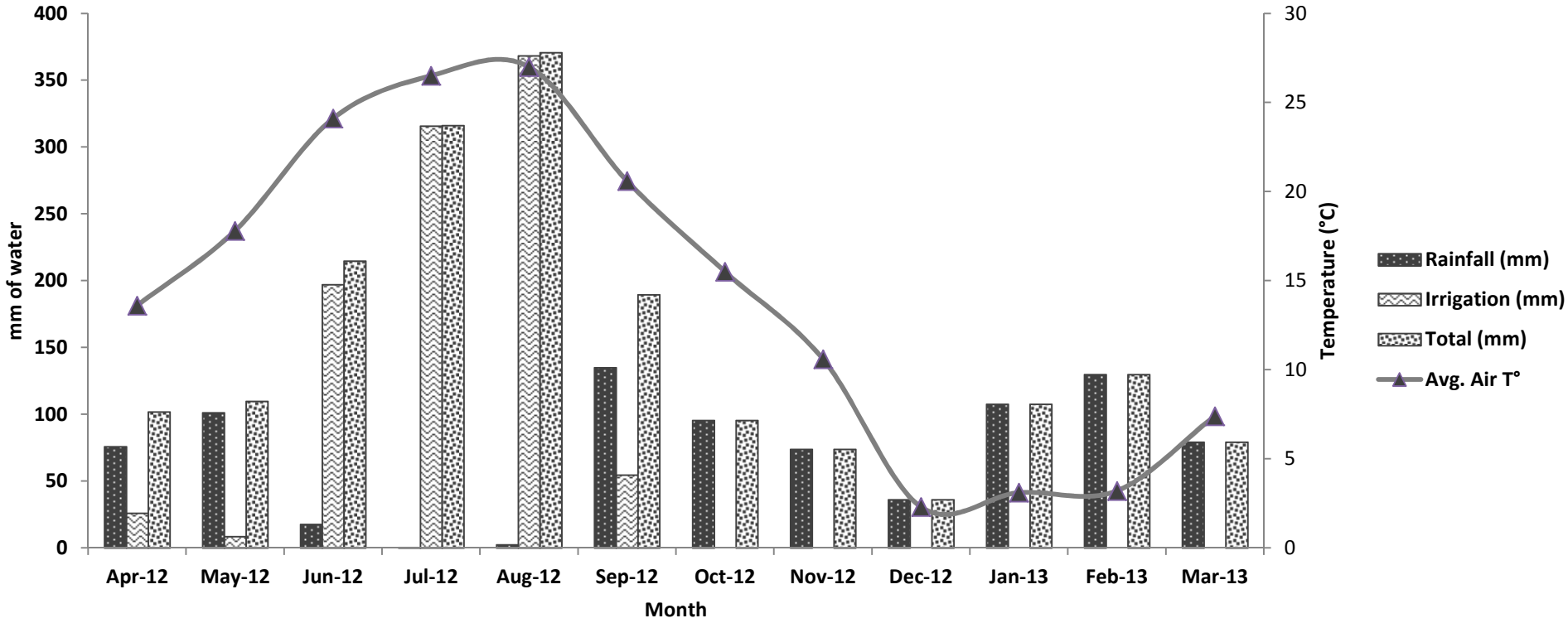


Figure 5.1. Rainfall, irrigation volumes and air temperature monthly recorded throughout the first year of experiment

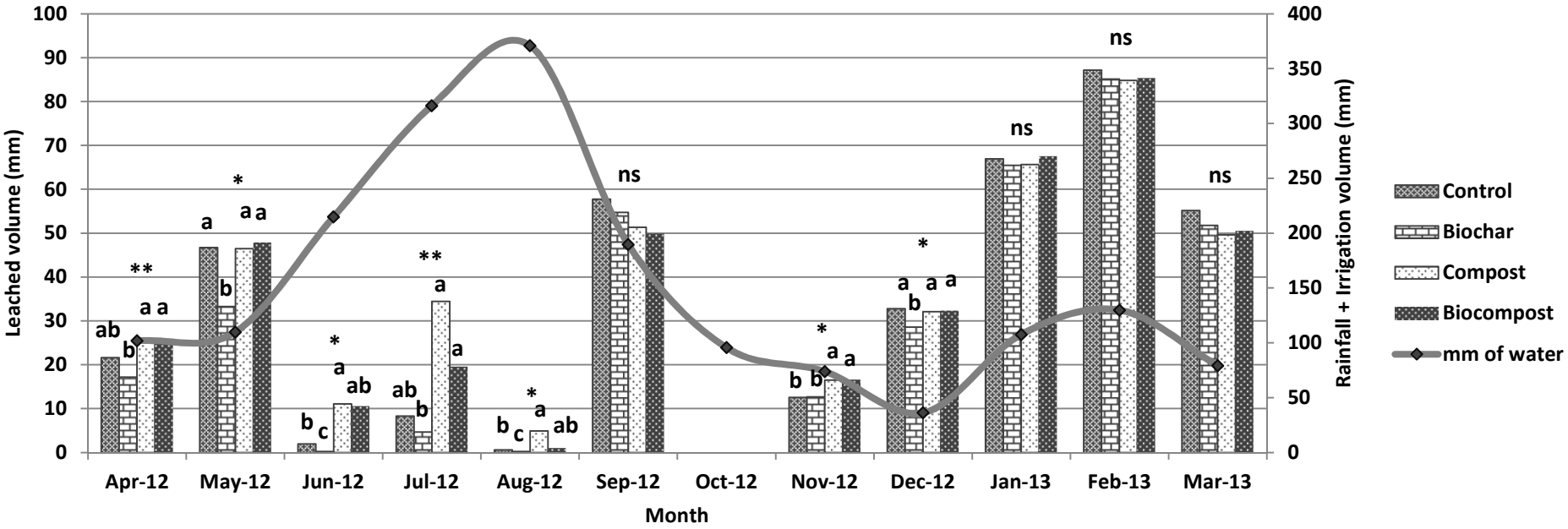


Figure 5.2. Effect of the amendment on the leached volumes and water supplied (rainfall + irrigation) to the lysimeters from April 2012 to March 2013

In October 2012 no leachate was collected due to insufficient rainfalls
ns, * and **: effect of treatment not significant or significant at P≤0.05 and P≤0.01, respectively. Within the same month, columns followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test)

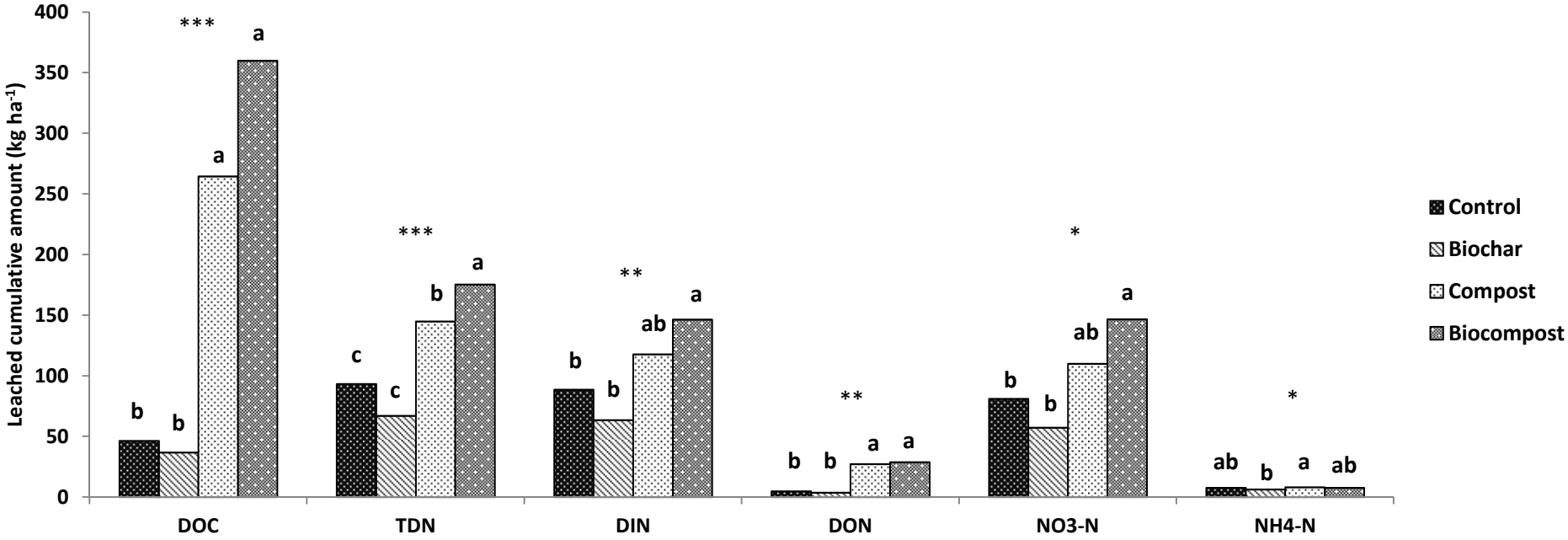


Figure 5.3. Cumulative amount (kg ha^{-1}) of dissolved organic C (DOC), total dissolved N (TDN), total dissolved inorganic N (DIN), total dissolved organic N (DON), Nitrate-N (NO_3^--N) and Ammonium-N (NH_4^+-N) in the leachate in 1 year (April 2012 - March 2013)

*, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01and P≤0.001, respectively. Within the same variable, columns followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test)

CHAPTER 6

CO_2 emissions partitioning, bacterial community profile and gene expression of *Nitrosomonas* spp. and *Nitrobacter* spp. in a sandy soil amended with biochar and compost

Abstract

This study evaluated the effect of soil-applied biochar, compost and their combination on soil properties, respiration partitioning, bacterial community profile and gene expression of *Nitrosomonas* spp. and *Nitrobacter* spp. A 2-year experiment was carried out on nectarine trees planted in March 2012 and grown in 0.496 m^3 pots filled with a sandy soil in which, with four replicates, the following strategies were compared with an unamended control: a) biochar (20 g fw kg^{-1}); b) compost (76.8 g fw kg^{-1}) and c) biocompost (same rates of the previous two strategies). Amendments were applied at planting and only unamended and biochar-amended soils were fertilized with mineral inputs. Soil pH, mineral N, soil temperature and soil water content (SWC) were periodically measured. Total soil respiration (R_{TOT}) was separated into soil organic-C derived (R_{SOC}) and rhizosphere (R_R) respiration by the trenching method. At the end of the experiment total C and N concentration of soil and recovered biochar fragments were measured. Total soil DNA was extracted from samples collected after 6 and 18 months and bacterial community analysis was carried out by PCR amplification and subsequent band identification (DGGE). Expression of nitrification key genes of Ammonia monooxigenase (AMO) and Nitrite oxidoreductase (NOR) and the relative abundance of specific bacterial community (*Nitrosomonas* spp. and *Nitrobacter* spp.) were determined by Real Time PCR on soil samples collected at 6, 12, 15, 16 and 18 months since amendments incorporation. Benefits on soil properties (i.e. SWC) and fertility (mineral N) were induced by the addition of compost which also promoted bacterial biodiversity, increased the relative expression of nitrification process related key genes. Furthermore, compost enhanced R_{SOC} likely due to the

stimulation of the microbial community by providing labile C sources. Conversely, changes due to the mere addition of biochar were negligible. However, biochar had no detrimental effects, rather it slightly promoted gene expression involved in the nitrification process. A synergistic effect between the two amendments emerged in the soil field capacity (FC), total soil C and N concentration and in the R_{SOC}, leading to a significantly higher cumulative evolution of CO_2. Although the source of the additional CO_2 rate remains uncertain, a priming effect induced by biochar on the labile compost-derived C-fractions is hypothesized. Compost reduced the relative richness of *Arthrobacter* spp. in soil while *Actinomadura flavalba*, *Saccharomonospora viridis*, *Thermosporomyces composti* and *Enterobacter* spp. were peculiar of the biocompost profile which increased band richness. Biocompost showed the significantly highest relative abundance of *Nitrosomonas* spp. and *Nitrobacter* spp. and both AMO and NOR key genes expression levels. The mixture of biochar and compost seems agronomical effective although environmental concerns (e.g. additional CO_2 emissions), require further investigations.

Keywords: Biocompost, soil respiration, *Nitrosomonas* spp., *Nitrobacter* spp., Ammonia monooxigenase, Nitrite oxidoreductase

6.1 INTRODUCTION

The incorporation of either pyrolized (biochar) or composted (compost) organic biomasses into agricultural or forested soils has raised interest worldwide since it has been heralded as a sustainable and cheap strategy to offset anthropogenic carbon (C) emissions, thus alleviate climate changes (Woolf et al., 2010; Hargreaves et al., 2008; Paustian et al., 1997), while ameliorating soil properties and growth conditions (Spokas et al., 2012; Verheijen et al., 2010) with positive implications in the reduction of social costs related to the recycling of organic solid and agri-food industry wastes, otherwise disposed. For instance, according to estimations reported by Woolf et al. (2010), annual net emissions of carbon dioxide (CO_2), methane (CH_4), and nitric oxides (N_2O) may decrease by 1.8 Pg CO_2-C equivalent and total emissions by 130 Pg CO_2-C equivalent over a century by implementing globally a sustainable biochar program. Biochar is a solid C-rich matrix obtained by biomass thermo-chemical decomposition under complete or partial exclusion of oxygen (pyrolysis). Once in soil, biochar has the potential to long-term increase soil C storage, maintain the balance of soil ecosystems and act as a soil nutrient-retaining additive (Spokas et al., 2012; Verheijen et al., 2010).

Compost is a stabilized amendment resulting from the biodegradation of organic wastes operated by various microorganisms under aerobic conditions. The use of compost in fruit tree ecosystems is considered environmentally safe and can be profitably adopted for its fertilization value (Sorrenti et al., 2012; Caballero et al., 2009) and as a mean to restore and enhance soil organic matter (OM) (Diacono and Montemurro, 2009). However, while agronomic benefits have been largely demonstrated, compost-induced long-term soil C sequestration potential is limited compared to biochar (Fischer and Glaser, 2012), since biogenic humic substances have a short residence times (Stevenson, 1994). The reason is that composted biomasses account for a large amount of easily-degradable C-containing substances which can be decomposed by soil microorganisms in the short-medium period, depending on the soil properties, temperature and compost quality (Thompson and Nogales, 1999). CO_2 and microbial biomass are the main products of such decomposition, together with a more stable humus-like organic matter fraction (Zwart et al., 1994) which is exposed to slower decomposition rates. Part of the

dissolved organic C (DOC) from compost can even leach to groundwater (Kaplan et al., 1995). In terms of soil C sequestration, the beneficial outcome from biochars are expected to last longer compared to other forms of soil organic C (SOC) pools due to the high aromatic nature of the biochar, which makes it relatively stable against whether biotic and abiotic decomposition (Spokas et al., 2012; Swift, 2001). Although scientific evidences demonstrated an initial increase in CO_2 emission as well as an increase in the leached amount of DOC after biochar addition to soils mainly as a consequence of the organic C breakdown and the release of biochar-derived inorganic C molecules, this is considered a short living effect, thereby negligible for SOC sequestration potential (Jones et al., 2011).

Combining biochar and compost in soil could offer a number of benefits compared to the incorporation of biochar and compost alone. Synergistic effects may include an enhancement of the nutrient use efficiency, the biological activation of biochar, the creation of humus and nutrient-rich substrates, together with a higher and long-term C sequestration outcome (Fischer and Glaser, 2012).

As a consequence of either biochar or compost incorporation in soils, a net increasing in soil microbial biomass has been recognized, with significant changes observed in the microbial community composition and enzymatic activities (Lorenz and Lal, 2014; Hu et al., 2014; Zhen et., al 2014; Ameloot et al., 2013; Biederman and Harpole, 2013; Anderson et al., 2011; Lehmann et al., 2011; Ros et al., 2006; Garcia-Gill et al., 2000). Mechanisms affecting soil biota upon biochar addition involve mainly sorption of allelopathic/inhibiting compounds or releasing of biologically active molecules, changes in pH and soil physical properties such as porosity, surface area, water holding capacity (WHC) and minerals (Ameloot et al., 2013; Lehmann et al., 2011). On the other hand, microorganisms can utilize a number of labile biochar-derived constituents as energy source (Cross and Sohi, 2011). With respect to compost, three main mechanisms have been indicated as responsible for the promotion of the biological activity in soil: a) the addition of easily degradable C-compounds, which represent valuable substrates for heterotrophic microorganisms; b) the provision of habitat and niche properties in soil (e.g. water and air balances, increase of specific surfaces) and c) the introduction of biota into soil via compost as inoculant (Amlinger et al., 2007;

Blume, 1989; Werner et al., 1988). However, compost-induced shift in microbial communities are not fully understood and in the case of biochar are only beginning to be explored (Lehmann et al., 2011). Furthermore, despite soil microorganisms play a central role in nutrient cycling and provide important ecosystem services (Costanza et al., 1987), specific effects induced by organic amendments in soils are poorly assessed. Most of the scientific evidences were obtained under lab conditions since outdoor is more complex and dynamic (due to biotic and abiotic interference such as exposure to freezing/thawing and drying/wetting cycles and the cultivation of crops).

As far as we know, no data are available on the combined effects of soil-applied biochar and compost on soil CO_2 fluxes, partitioning into root-derived and soil organic C-derived components and on microbial diversity and efficiency. Elucidate mechanisms that govern how the addition of biochar, compost and their mixture to soils may affect ecological and agronomical aspects are therefore of crucial importance in the conjecture that these could be widely adopted in the near future. In particular, scientific acquisitions about the effect of such amendments on specific groups of microorganisms involved in key biochemical cycles in soil (i.e. nitrogen-N) appear of primary importance.

In this context, this study focused on the soil respiration partitioning, bacterial community profile, gene expression of *Nitrosomonas* spp. and *Nitrobacter* spp. abundance and efficiency and soil properties (e.g. pH, WHC, mineral N availability) in nectarine trees grown on a sub-alkaline sandy soil amended with biochar, compost and their combination. We tested the hypothesis whether or not biochar and compost may synergistically act, shifting soil CO_2 fluxes partitioning, promoting microbial community changes and altering soil related physical and biological processes. This research is part of a larger study which included the effect of the amendments on the soil leaching, crop growth and root physiology.

6.2 MATERIALS AND METHODS

6.2.1 Experimental conditions

A 2-year experiment (2012-13) was carried out outdoors at the experimental station of the University of Bologna (44°54' N, 11°41' E, 36 m a.s.l.) on 1-year old nectarine trees (*Prunus persica* (L.), Batsch) of the cv. Big Top grafted on Adesoto (Puebla de Soto 101, *Prunus insititia* (L.), Bullace) grown in 0.496 m^3 pots and filled with 503 kg ea. of a sandy soil, which main physical-chemical characteristics are summarized in table 6.1.

In Spring 2012, one tree per pot was planted, trained as slender spindle, covered with a shade netting (in summer) which allowed a shadow of 31% compared to full sun (measured in summer at noon on a sunny day), watered from May to September by microirrigation (4 drippers per plant of 2 L h^{-1} ea.) to return the evapotranspiration (ETo) rate as estimated by a class A evaporation Pan and the specific crop coefficient (Kc) for nectarine, whereas weeds were hand-removed.

The climate is temperate sub-continental with cold winters and humid and warm summers.

Picture 6.1. View of the pots that were arranged in a single row, spaced 0.50 m between each other and N-S oriented

6.2.2 Experimental design and treatments

We compared the following soil-applied strategies arranged in a complete randomized block design (with 4 replicates): a) biochar (20 g fw (fresh weight) kg^{-1}) equal to 87.4 t fw ha^{-1} (considering a soil incorporation up to 0.35 m depth and a specific soil weight of 1.248 t m^{-3}); b) compost (76.8 g fw kg^{-1}); d) biochar+compost (biocompost from now on) (20 and 76.8 g fw kg^{-1} of biochar and compost, respectively). Unamended pots were included in the experimental design as control. Amendments were homogenised with the soil and applied only at planting. Unamended and biochar-amended soils received 41.7, 9.3 and 6.9 g pot^{-1} of N, P and K, respectively in the first season and 62.4, 12.0 and 22.9 g pot^{-1} of N, P and K, respectively, in the second season using commercial urea (46% N), ammonium-nitrate (NH_4NO_3) (27% N) and a mixture of NPK (14-25-5) + microelements as a source of nutrients. Fertilizers were applied regularly by fertigation from petal fall until the end of the vegetative seasons. Compost-based amended soils did not receive chemical sources of fertilizers while the same amount of tap water was supplied in coincidence with fertigation events.

Biochar was obtained in a commercial charcoal kiln by slowly pyrolysing (550°C) a mixture of chipped peach and grapevine hardwood (in prevalence), while compost is the result of a 3-month stabilization biological decomposition of pruning materials from urban ornamental trees and garden management (6.5%) mixed with organic municipal wastes (85%) and agro-industrial organic residues (8.5%), under aerobic conditions. Main physical and chemical characteristics of biochar and compost used in the experiment are summarized in table 6.2 and 6.3, respectively.

6.2.3 Soil pH and KCl extractable N mineral forms

Every 2 months, one sample per pot was obtained by homogenizing 4 soil cores collected at 0.05-0.30 m depth. A subsample was oven dried (105°C) and grinded (2 mm mesh), then 10 g were added to 25 mL of deionized water (d-H_2O) and shaken 1 h at 95 rpm by an orbital shaker. The pH was measured on the filtered supernatant with a pH-meter (BasiC 20, Crison, Barcelona, Spain) under continuous stirring.

To evaluate soil N mineral content, 10 g of fresh soil were extracted by a 2 M KCl solution at a ratio of 1:10 (w w^{-1}). Samples were shaken 1 h at 95 rpm by an orbital shaker, filtered (Whatman 42) and analyzed for nitrate-N (NO_3^-- N) and ammonium-N (NH_4^+-N) concentration by a continuous flow autoanalyser (AA-3, Bran+Luebbe, Norderstedt, Germany). Soil moisture content (w w^{-1}) was evaluated gravimetrically by oven drying at 105 °C representative subsamples.

6.2.4 Total C and N content of soil and aged biochar fragments

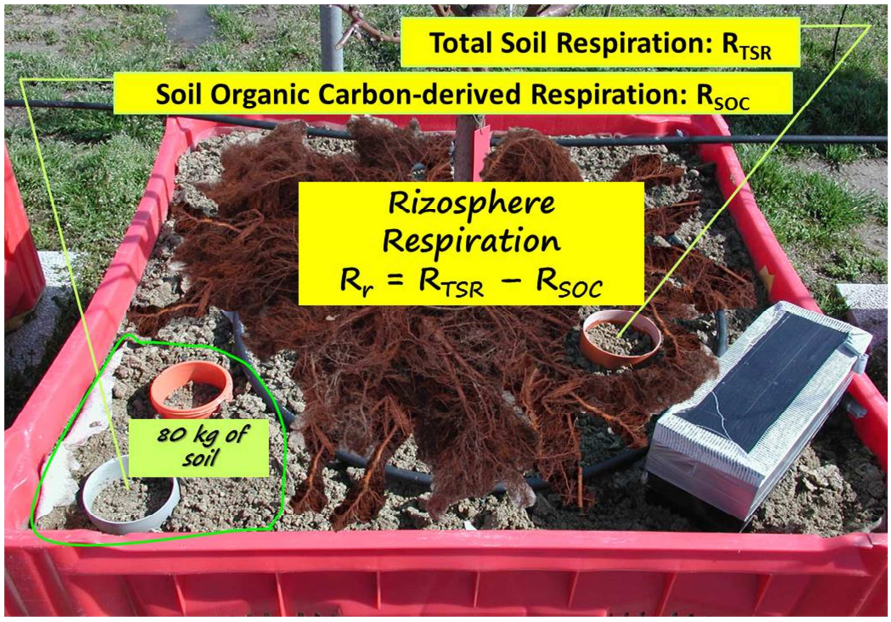
Subsamples of the soils collected at the end of the experiment (September, 2013) and used to extract mineral N forms were oven dried and manually pulverized in a mortar, then 13 (±0.5) mg per sample were used for total C and N determination.

In October 2013 (after 19 month from trial establishment), we randomly recovered about 30 biochar fragments per sample from the pots amended with biochar and biocompost by forceps, avoiding manual contact and any physical damage to the particles. At the same time, biochar fragments never field-applied, hermetically stored in plastic bags and maintained in a dry and dark place, were included as control (termed here as "fresh"). Particles, including fresh fragments, were first dried at 50 °C for few days, gently sieved (1-mm) to remove exceeding soil particles and then the surface was gently cleaned with a soft brush and sparingly rinsed twice with d-H_2O to remove adhering soil from the surface. Fragments were oven-dried at 50 °C, manually milled using a mortar and then 3 mg for total N and 0.1 mg for C determination were weighted. Both soil and biochar samples were analyzed via catalytic combustion analysis (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA) at 2.33 mV voltage. Retention time was 1.21 and 1.78 min for N and C, respectively. Data were compared with external calibration curves at 9 points (r^2 > 0.9995) obtained by a high-purity acetanilide standard (Costech Analytical Technologies Inc., Valencia, CA).

6.2.5 Soil respiration partitioning, temperature and water content (SWC)

Total soil respiration (R_{TOT}) was measured in one partially buried (0.02 m) cylinder-shaped PVC collar per pot (Ø of 0.10 m and 0.06 m height) using an infrared gas analyzer (EGM 4, PP Systems, Amesbury, MA, USA) equipped with a

closed dynamic chamber (SRC 1, PP Systems Amesbury, MA, USA) which allows to measure soil CO_2 efflux (g CO_2 m^{-2} h^{-1}) from the collars that were never disturbed or removed from soil during the entire experiment time course. Air in the dynamic chamber was at ambient CO_2 concentration, modulated automatically by circulating air through a soda lime column between consecutive measurements; collars were adapted to tightly fit the chamber. Measures were initiated when CO_2 concentration remained constant, usually between 70 and 80 sec after placing the chamber onto soil collars. R_{TOT} was partitioned in soil organic-C derived respiration (R_{SOC}) and rhizosphere respiration (R_R) by the trenching method adapted from Kuzyakov and Larionova (2005). To this end, one trench per pot was set up at transplanting by isolating about 16% of the total soil volume (about 80 kg pot^{-1}) by a geotextile canvas ensuring free circulation of gases and solutes while preventing root ingrowths. One PVC collar per pot, as above described, was placed on the surface of the isolated soil volume in order to measure the R_{SOC} without the interference of the roots.



Picture 6.2. Schematic illustration of the soil CO_2 respiration partitioning

In all pots, trenches and collars were identically oriented and set up at the same distance from drippers and trunks. In each pot, R_{SOC} and R_{TOT} were measured consecutively in the same day between 10:30 h and noon, at about 20 days interval. For each sampling, the R_R rate was estimated as the difference between R_{TOT} and R_{SOC}, according to the following equation:

$$R_R = R_{TOT} - R_{SOC}$$

R_R represents the autotrophic respiration component while R_{TOT} and R_{SOC} are the rates of total and SOC-derived respiration, measured in non-trenched and trenched soil volumes, respectively. However, R_R rate was estimated from the second growing season. After each soil respiration measure, the topsoil temperature inside each collar was measured by a portable digital thermometer (TR-50303, Forlì, Italy) coupled with a PT-100 probe (TR, Forlì, Italy) which was inserted at 0.03 m depth and left until stabilize. Later on, the top-soil water content (SWC) (w w^{-1}) was measured gravimetrically by oven drying at 105 °C 4 representative soil cores sampled at 0-0.03 m depth and close to the collars.

For each sampling, R_{SOC} was parameterized to soil temperature using the following exponential model, as described by Ventura et al. (2014):

$$R_{SOC} = R_{10}e^{c(T-10)}$$

R_{SOC} represents the heterotrophic soil respiration flux as measured in the collar, T is the soil temperature as measured at 0.03 m depth and R_{10} and c are coefficients estimated by a nonlinear regression statistical procedure.

In addition, we calculated the apparent sensitivity of the R_{SOC} to soil temperature, expressed as Q10 ($=e^{10c}$).

We also determined, for each sampling date, the influence of the SWC on the R_{SOC} deprived of the interference of the soil temperature by estimating a value of R_{10} according to the following equation:

$$R_{10} = R_{SOC}/e^{c(T-10)}$$

R_{SOC} is the measured SOC-derived respiration rate and c is a coefficient set at 0.0693 corresponding to a Q_{10} of 2.5 as described by (Bååth and Wallander, 2003). R_{10} is an estimate of the soil respiration flux assuming a stable soil T of 10 °C. Obtained values were then plotted against SWC.

6.2.6 Total soil DNA extraction

In September 2012 and after 1 year (September 2013), one soil sample per pot was obtained by homogenizing 4 soil cores collected at 0.05-0.20 m depth by a push-in hand soil sampler which was cleaned between two consecutive sampling. Root from soil samples were carefully removed and then frozen at -80°C to await analyses. To this end, total community DNA was extracted from 250 mg of soil using the PowerSoil DNA kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions and modified by Gaggìa et al. (2013). Briefly, 5 µL of mutanolysin (100 U mL^{-1}, Sigma-Aldrich) and 195 µL of lysozyme (50 mg mL^{-1}, Sigma-Aldrich) were added to the soil powder in the bead solution supplied with the kit. The soil suspension was then incubated at 37 °C on a rotary shaker for two hours, prior to chemical (with SDS-containing solution supplied with the kit) and mechanical (bead beating on vortex at maximum speed for 10 min) cell lyses. DNA was eluted with 70 µL of 10 mM Tris-HCl pH 8.0. The purity and quantification of extracted DNA was determined by measuring the ratio of the absorbance at 260 and 280 nm (Infinite® 196 200 PRO NanoQuant, Tecan, Mannedorf, Switzerland). Extracted DNA was stored at -20 °C.

6.2.7 Polymerase Chain Reaction (PCR)

PCR amplification was performed on control and amended soil samples using 50 ng of extracted DNA as a template with the universal bacterial primer pair 968F with a 40-bp GC clamp attached to its 5'end (Heuer et al., 1997) (5'-cgcccggggcgcgccccgggcggggcgggggcacgggggg-aacgcgaagaaccttac-3') and 1378R (5'- cggtgtgtacaaggcccgggaacg-3'). The 50 µl PCR reaction contained 1.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems), 5 µL of 10X PCR Gold Buffer (Applied Biosystems), 200 µM of each deoxynucleotide triphosphate

(Fermentas GmbH, St.Leon-Rot, Germany), 1.50 mM $MgCl_2$ (Fermentas), 0.5 μM of each primer (Eurofins MWG Operon, Ebersberg, Germany), 0.5 mg mL^{-1} bovine serum albumin (Fermentas), 2 μL DNA template (50 ng uL^{-1}) and sterile MilliQ water. A touchdown thermal cycle program was used to prevent non-specific amplification, which included an initial denaturation at 95 °C for 2 min followed by 9 cycles each of denaturation at 95 °C for 1 min, 60 °C with 0.5 °C decrease per cycle for 1 min, and extension at 72 °C for 2 min, and completed with 20 additional cycles when annealing temperature reached 55 °C. A final extension at 72 °C for 10 min was included at the end of the cycles before holding at 4 °C. The size and amount of the PCR products were estimated by analysing 2 μL samples by 1.5% agarose gel (w v^{-1}) electrophoresis and ethidium bromide staining.

6.2.8 Denaturing gradient gel electrophoresis (DGGE) and band identification

The bacterial community analysis was carried out by DGGE, according to Muyzer et al. (1993), using a DCode System apparatus (Bio-Rad, Richmond, CA, USA), employing 7% polyacrylamide gels with a denaturing range of 35–55% The electrophoresis was run at 55 V for 16 hours at 60°C. Gels were stained in a solution of 1X SYBR-Green (Sigma–Aldrich) in 1X TAE for 20 min and their images captured in UV transillumination with Gel Doc™ XR apparatus (Bio-Rad). Selected bands were cut from the gel with a sterile scalpel and DNA was eluted by incubating overnight the gel fragments in 50 μl of sterile deionised water at 4 °C. 2 μl of the solution were then used as template to re-amplify the band fragments with the same PCR condition described above. After amplification and repeated DGGE, purity and comobility with amplified DNA obtained directly from soil samples was assured. After purification, PCR products (obtained by using primers without the GC-clamp), representing single bands, were sent for sequencing (Eurofins MWG Operon). Sequence chromatograms were edited and analysed using the software programs Finch TV version 1.4.0 (Geospiza Inc., Seattle, WA, USA). GenBank DNA sequences with the highest similarity to those represented by the DGGE bands were identified using the BLAST alignment tool (http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul et al., 1997).

6.2.9 Expression of key genes of Nitrosomonas spp. and Nitrobacter spp. in soil

In September-12, March-13, June-13, July-13 and September-13, one soil sample per pot was obtained by homogenizing 4 cores collected at 0.05-0.30 m depth, sieved using a 2 mm sieve to remove root residues, while biochar particles (< 2mm) were not excluded through sieving then frozen at -80°. RNA and DNA were extracted according to the methodology described by Hurt et al. (2001) using nucleic acid binding column from Total soil DNA Extraction Kit (MACHEREY-NAGEL GmbH & Co, DE) and Total RNA extraction kit (Norgen Biotek, CA, USA). DNA and RNA quality and quantity were assayed at 230, 260 and 280 nm absorbance (λ) by NanoDrop 1000 Spectrophotometer (ThermoScientific, Wilmington, USA), and agarose gel staining with ethidium bromide ($C_{21}H_2OBrN_3$).

6.2.10 Genes expression analysis

Expression of nitrification key genes Ammonia monooxigenase (AMO) and Nitrite oxidoreductase (NOR) and the relative abundance of specific bacterial community (*Nitrosomonas* spp. and *Nitrobacter* spp.) were determined by Real Time PCR. The primers used were: Amo gene, AmoA Forward, and AmoA Reverse for Amo (Rotthauwe et al., 1997); NxrA forward and NxrA reverse for Nir (Wertz et al., 2008); NITISR forward and NITISR reverse for *Nitrobacter* spp. quantification (Hawkins et al., 2008); 16s forward and reverse 16s for *Nitrosomonas* spp. quantification (Lim et al., 2008). Retrotranscription of purified RNA was performed by using the cDNA First-Strand Synthesis kit (Life Technologies, Rockville, MD, USA) according to the manufacturer's recommendations. Gene expression was determined using a StepOne Plus Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) with a SYBR green-based assay. Each reaction was performed in 10 μL, containing 5 μL of Power SYBR Green Master Mix 2×, 70-100 mM of each primer, 3 μL of a 1:4 dilution of the cDNA and PCR-grade water. Reactions were performed in triplicate and incubated 2 min at 50 °C then 5 min at 95 °C. Samples were then subjected to 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Data were collected at each annealing step. Gene expression was determined by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001)

and expressed as Fold Change (FC) relative to a housekeeping gene, (16s primer set for AMO and NITISR primer set for NOR). Relative quantitation of bacterial abundance was performed as previously described using as template total soil DNA. Data were expressed as population Fold Change (FC) related to the total DNA extracted from the unamended soil according to a standard curve (Larionov et al., 2005).

6.3 STATISTICAL PROCEDURES

Data of soil pH, soil N mineral forms, trends of soil CO_2 emissions, soil temperature and SWC were submitted to repeated measures analysis of variance (ANOVA) with 4 replicates using PROC MIXED (Littell et al., 1998) with a compound symmetry covariance structure, according to a complete randomized experimental design.

Other data were submitted to the analysis of variance according to a complete randomized design. When analysis of variance showed a statistical effect, means were separated by using Tukey's HSD Test (at $P \leq 0.05$). Statistical analyses were performed by using SAS software (SAS Institute Inc., Cary, NC, USA). Data relative to the Real Time PCR were analyzed by the Stat software (STATISTICA version 5.0, Statsoft Inc. 1995, Tulsa, OK, USA) and means were separated by using the Student–Newman–Keuls (SNK) test (at $P \leq 0.05$). Homogeneity of variance was checked using Levene's test before analysis. Dependence models of R_{SOC} with soil temperature and R_{10} with SWC were estimated with non-linear regression analysis, respectively. Pearson correlation coefficient between soil T and R_{SOC} was calculated.

Band richness on the DGGE profiles was analysed with the one-way ANOVA with the GLM procedure of SAS to evaluate difference among treatment over time. A post-hoc analysis with Duncan Test has been performed to compare the different groups using Bonferroni adjusted alpha level ($p<0.002$). DGGE patterns were digitally processed using the GelCompar II software 6.6 (Applied Maths, Kortrijk, Belgium). Comparison and cluster of DGGE profiles were carried out using the unweigthed pair-group method with the arithmetic average (UPGMA) clustering algorithm based on the Pearson product-moment correlation coefficient and resulted in a distance matrix. Multidimensional scaling (MDS) and principal components analysis (PCA) were carried out by the Gel Compare II software.

6.4 RESULTS

6.4.1 Soil pH and N mineral forms

Soil pH values fluctuated in the sub-alkaline range, between 7.52 and 8.06 (Tab. 6.4) and, unless in July-12, it was significantly decreased by compost and biocompost (Tab. 6.4), while comparable values were measured between unamended and biochar-amended soils (Tab. 6.4).

Treatments did not affect soil inorganic N in June and July-13 (Tab. 6.5) and NH_4^+-N concentration resulted unaffected in September, November 12 and May 13 (Tab. 6.5). In the other sampling, NH_4^+-N concentration was increased in compost-treated soils, either with or without biochar (Tab. 6.5). A similar trend was also observed for soil NO_3^--N concentration (Tab. 6.5), with the exception of May-13 when soils that received mineral fertilizer sources (unamended and biochar-amended) showed an increased NO_3^--N concentration compared to compost-based treatments (Tab. 6.5). Only in one sampling (i.e. November 12), soil amended with biochar increased the concentration of NO_3^-- N compared to control soil (Tab. 6.5), while in January and March 13, independently of the strategy, the NO_3^-- N concentration in the soil was undetectable (Tab. 6.5.). The NO_3^-- N fraction in soil was predominant during summer while it appeared either low or even lower than the detection limit (dl) during winter (Tab. 6.5).

6.4.2 Total C and N content of soil and aged biochar fragments

Biocompost significantly increased soil C and N content compared to other treatments (Tab. 6.6), while compost alone showed intermediate values of soil N content between biocompost and the remaining strategies. C and N content in biochar and unamended soils were statistically similar (Tab. 6.6). The environmental exposure did not affect C concentration in biochar fragments while significantly increased that of N, independently of the presence of compost (Tab. 6.6).

6.4.3 Soil respiration partitioning

6.4.3.1 Soil Organic C-derived respiration (R_{SOC}) and soil °T dependence

The R_{SOC} (heterotrophic respiration) flux followed a seasonal pattern following the soil T trend. R_{SOC} increased from spring to summer while remained negligible, than it was negligible (0.2 g CO_2 m^{-2} h^{-1}) in winter (Fig. 6.1). Strategies significantly changed R_{SOC} (Fig. 6.1) and, unless in winter, CO_2 flux was increased by compost with values up to 3-fold greater than unamended and biochar treated soils (Fig. 6.1). In addition, the mixture of the two amendments, statistically promoted R_{SOC} in 12 out of 29 sampling compared to the mere addition of compost (Fig. 6.1). The trend of the biochar and unamended soil CO_2 fluxes was often overlapped (Fig. 6.1), without significant differences.

Throughout two seasons, the trend of the cumulative R_{SOC} evolution was statistical equivalent between biochar and control soils, while values of biocompost and, to a less extent of compost, were significantly increased reaching at the end of the experiment values greater by 4 and 3-fold, respectively (Fig. 6.2).

Data of R_{SOC} fitted with soil T according to an exponential model (Fig. 6.4) and R^2 was 0.82, 0.83, 0.77 and 0.81 for control, biochar, compost and biocompost, respectively. Treatments did not induce differences in the apparent sensitivity of R_{SOC} to soil temperature (Q10) which ranged between 3.14 and 3.74 (Fig. 6.4). Soil moisture was not correlated to R_{SOC}, even after data normalization (data not show).

6.4.3.2 Total soil respiration (R_{TOT})

Values of R_{TOT} were higher than those of R_{SOC}, and followed a similar trend (data not shown). In details, no differences were observed between biochar and control, while CO_2 fluxes were significantly increased by compost, independently of the mixture with biochar (data not shown).

6.4.3.3 Rhizosphere respiration (R_R)

R_R represents the autotrophic respiration estimated as the difference between the previous components (R_{TOT} - R_{SOC}). The contribution of R_R to the R_{TOT} was unaffected by soil amendments unless in May and June 13, when values were

significantly decreased by compost compared to the other two treatments (data not shown).

6.4.4 Soil temperature and water content (SWC)

Amendments strategies did not affect soil temperature (data not shown) which fluctuated between -0.79 (± 0.03) in December 12 and 29.7 °C (±0.04) in July 13. Water content in the top soil (0-0.05 m) was increased by biocompost and, to a less extent, by compost, while biochar did not differ from untreated control (Fig. 6.3). Likewise, this trend was confirmed considering the deeper soil profile (5-0.30 m). However, biochar mixed with compost revealed the highest values (Tab. 6.7), while the addition of biochar and compost alone induced comparable effects. The addition of biochar and compost to the soil increased the SWC in two and four sampling (out of 10), respectively (Tab. 6.7).

6.4.5 Microbial community profiles and band identification

In order to analyze the influence of the amendments on the bacterial community structure, PCR–DGGE fingerprints targeting the 16S rDNA fragment were performed at two sampling time carried out six months after the amendments incorporation (September 2012) and then one year later (after 18 months, in September 2013). Band richness was affected by sampling time and treatment (Fig. 6.5). Without interaction between the two factors, band richness was significantly higher in the last sampling, while biocompost significantly promoted band richness and, to a less extent, also by biochar compared to the other strategies (Fig. 6.5). Compost amended soil revealed the same band richness as the unamended soil (Fig. 6.5).The cluster analysis of the 16S rDNA banding profiles generated by DGGE showed high similarity (over 80 %) between banding profiles belonging to the same treatment and sampling time (Fig. 6.6). However, a first division emerged between samples from control and biochar soils sampled in 2013 compared to the other treatments at both sampling time, with a similarity lower than 49.8 % (Fig. 6.6). A second clustering level separated the sampling of 2012 (independently of the treatment), from biocompost and compost in 2013 (59.1 % similarity) (Fig. 6.6). With few exceptions, further divisions within each cluster allowed the

discrimination between treatments and therefore the generation of representative individual clusters, indicating homogeneity within treatment and/or sampling time. The MDS plot (Fig. 6.7) gathered the combination of treatment and sampling in three clusters, corresponding to a) biocompost and compost (2013); b) control and biochar (2013) and c) all treatments at the first sampling (2012) (all treatments). Results of the PCA analyses are shown in Fig. 6.8. The total variance explained by the first two axes was 57.6% (20.4% axis 1 and 37.2% axis 2). The PCA did not separate completely the different groups, although the clustering was comparable to the dendrogram and the MDS. However, PCA divided, along axis 2, samples collected in September 2012 from those sampled 12 months later (Fig. 6.8).

Figure 6.9 shows band excision from DGGE analyses carried out on soil samples at the end of the experiment. The similarity of band sequences ranged from 94 to 100% compared with those available in the GenBank database (Tab. 6.8). The most relevant band (# 6) detected in all samples with a higher intensity in unamended and biochar-treated soils was identified as *Arthobacter* spp., *Gloeobacter kilaueensis* (band 3) is particularly evident in biocompost sampled in 2013. Remaining bands are peculiar of soil amended with compost profiles, either with or without biochar, and ascribed to *Planifilum fimeticola* (band 1), *Actinomadura flavalba* (band 5), *Saccharomonospora viridis* (band 7), *Thermosporomyces composti* (band 8) and *Enterobacter* spp. (band 9 and 10).

6.4.6 Effect of the amendments on *Nitrosomonas* spp. and *Nitrobacter* spp. abundance and efficiency

Relative gene expression activity (as measured by gene expression levels of AMO and NOR) and relative *Nitrosomonas* spp. and *Nitrobacter* spp. abundance (as measured by gene expression levels of specific genes) were stimulated, at each sampling, by biocompost and, to a less extent by compost, compared to the unamended control and biochar (Fig. 6.10).

On the contrary, the addition of biochar to the soil slightly increased the abundance of *Nitrobacter* spp. only in the sampling of September-13, while no differences were observed for *Nitrosomonas* spp. population. Likewise, AMO and Nir and

ammonia gene expression were significantly promoted by biochar in one and 2 sampling time, compared to the unamended soil (Fig. 6.10).

6.5 DISCUSSION

6.5.1 Effect of biochar and compost on soil properties

The most remarkable effects on soil properties were induced by the addition of compost whereas changes due to the mere addition of biochar were not noticeable compared to the unamended control. However, the two amendments synergistically interacted in the SWC and total soil C and N content.

Although a pH increase has been indicated among the first changes on soil properties upon biochars addition (Atkinson et al., 2010; Major et al., 2010) and despite the alkaline pH of our biochar, pH values of biochar-treated soil did not change compared to the unamended soil. The sandy soil we used was naturally alkaline (pH=8.08) thus, we believe that biochar-induced changes on this parameter would require much higher rates than we adopted in our study. Likewise, Ventura et al. (2014) did not observe significant changes in soil pH after few years upon the addition of the same biochar (at a rate of 10 t ha^{-1}) used in this study in an apple orchard grown in a Haplic Calcisol sub-alkaline soil. Unlikely the biochar-induced liming effect will appear after several years from its application because weathering processes occurring on the biochar fragments exposed to the environment, in particular the development of carboxylic acids functional groups will lead to a decrease in the concentration of basic sites on the biochar surface (Yao et al., 2010; Cheng and Lehmann, 2009) which may reduce the pH of aged biochar. From an agronomical point of view, this observations could result positive since one of the unwanted effect in alkaline-calcareous soils would be a further pH increase due the alkaline properties of much of the current biochars (Sohi et al., 2010) because high soil pH hinders the availability of micronutrients (i.e. Fe, Mn, Zn) for plant uptake.

Conversely, compost decreased the soil pH likely due to the humic compounds, mainly supplied as humic and fulvic acids with compost which are developed during the composting process. These substances are an heterogeneous mixture of polyacidic compounds containing free and bound phenolic OH groups, quinone structures, N and oxygen (O) as bridge units and carboxylic (-COOH) groups, variously placed on aromatic rings. Among the properties of the humic acid fraction, there is the considerable buffer capacity in a wide pH range which arises

from the dissociation of their acidic functional groups (Ceppi et al., 1999). This may contribute to explain why the addition of biochar mixed with compost was also inconsequential on soil pH.

As expected, the addition of compost significantly increased the SWC in the topsoil and in the 0.05-0.30 m layer. An even greater SWC is observed in the soil amended with biocompost suggesting a positive synergism between the two amendments. Literature widely describes an increasing of SWC and field capacity (FC) after compost application (Evanylo et al., 2008; Liu et al., 2007; Tejada et al., 2006) and mechanism is mainly ascribed to the ability of OM to take up and retain water up to 20 times its own weight (Reicosky, 2005) since OM in soil increases the number of micropores and macropores either by contributing to the stability of soil aggregates through the bonding or adhesion properties of organic materials or by creating favorable living conditions for soil organisms.

Interestingly, the combination of biochar and compost further enhanced SWC. We suppose that compost and biochar, acting through different mechanisms, led to an additive effect and, as a result, the soil FC shifted up. This interaction suggests that mixing biochar and compost may represent an effective strategy to save water in agricultural soils while assuring adequate SWC for crop growth. Although results are sometimes conflicting (Verheijen et al., 2010), changes on SWC upon biochar addition were documented (Baronti et al., 2014; Basso et al., 2013). Biochar did not evidently enhanced SWC compared to the unamended soil throughout the experiment. This indicate that compost is more effective in improving SWC compared to biochar in a sandy soil (at our rates) and that likely the rate of biochar we tested in this experiment was not enough to induce statistical modifications.

The two amendments interacted with the total soil C and N concentration at the end of the experiment, which were 2 and 5 fold higher than the unamended control, respectively. Compared to other strategies, biocompost almost doubled the soil C content. Different mechanisms can be proposed as possible explanation of such response: a) the interaction between the two amendment promoted a faster humification process in soil leading to the formation of higher amount of stable humus-like substances within the experiment timescale; b) since biochar is capable of sorbing C-containing compounds (Barnes et al., 2014), part of the DOC (likely

from compost) was retained within the soil-biochar matrix; c) the two amendments promoted soil microbial biomass hence C immobilization; d) all or part of the previous mechanisms occurred simultaneously at different rates. This synergistic beneficial effect suggests positive implications in the C sequestration potential and soil fertility. On the other hand, the mere addition of biochar and compost did not significantly contributed to increase the soil C pools 18 months after their incorporation. Likely, a longer time could be required for either biochar to be fragmented into finer particles as a consequence of physical, chemical and biological degradation (Rutigliano et al., 2014) or for compost to originate stable humic compounds and enter as a part of stable soil C pool fractions.

Likewise, a synergistic effect between biochar and compost was observed on the soil total N concentration, which resulted significantly increased compared to compost alone. On the contrary, the addition of biochar alone did not change total N concentration in soil at the end of the experiment. We suppose that, acting as an N retaining-additive in soil, biochar held N supplied with compost which availability was higher that supplied by mineral sources. This may also indicate an increased N availability for plant uptake. Nevertheless, the concentration of the soil inorganic N (NO_3^--N and NH_4^+-N) was increased, with the exception of the sampling of May-13, by the addition of compost. On the other hand, it is also supposable that the extra N rate was immobilized into the soil under organic forms, hence unavailable to plants. However, data obtained from the same experiment revealed a significantly higher amount of total N (TDN) recovered in the 1-year cumulative leachate from the soil amended with biocompost (data not shown) compared to compost alone. This difference was ascribed to the inorganic N (DIN) fraction, in particular to the NO_3^--N form (data not shown). Based on these observations, we speculate that biochar and compost synergistically interacted affecting N cycle into the soil. This effect lead to an increase of the TDN in soil, likely as a consequence of a promoted nitrification process which was not correlated to the indirect effect of the compost-induced changes in soil pH. The effect seems to be related to the shift of the nitrifying bacterial community in soil (see further discussion).

6.5.2 Effect of the amendments on soil respiration partitioning

6.5.2.1 Biochar did not change soil organic C-derived respiration (R_{SOC})

The mere amendment with biochar did not alter R_{SOC} flux. This is in line with the total C concentration of the biochar fragments at the end of the experiment that was not affected by 18-month of environmental exposure, contributing to strengthen the concept of biochar as a potential C-sequestration strategy. Nevertheless, the effect of soil-applied biochar on soil respiration remains ambiguous. Incubations studies have measured a biochar-derived C lost up to 0.79% of the total C concentration within 2 months (Hamer et al., 2004). Previous studies indicated a greater soil respiration following addition of low temperature (250°C and 400°C) biochars. This response was attributed to both biotic and abiotic processes (Cross and Sohi 2011; Jones et al., 2011) such as microbial decomposition of the labile biochar soluble C fractions (Zhang et al., 2012), desorbable compounds (Borchard et al., 2014; Bruun et al., 2012; Jones et al., 2011) or an abiotic release of C (Zimmerman, 2010). On the contrary, biochars produced from woody materials and crop residues pyrolized from 450°C to 1100°C did not report effects on C mineralization (Borchard et al., 2014; Zavalloni et al., 2011; Steinbeiss et al., 2009; Spokas and Reicosky, 2009). Similarly, the incorporation of a suite of biochars produced from spent coffee grounds, wood pellets and horse bedding compost at 700ºC did not alter soil CO_2 emissions (Zhang et al., 2014). This is supported by Karhu et al. (2011) and Van Zweiten et al. (2010) who described no net increase in CO_2 evolution with birch (*Betula* ssp.) charcoal and 9 different biochars, respectively indicating the formation of recalcitrant aromatic structures during thermal degradation (Bruun et al., 2012) as possible explanation. Finally, a decreased CO_2 flux from a soil amended with biochars (>525°C) was attributed to a toxic effect induced on microbes and to the adsorption of DOC by biochar (Dempster et al., 2012), while Case et al. (2013) indicated that a stabilization of SOM might have occurred in the presence of biochar in a field experiment to explain the suppression of soil respiration as a consequence of biochar incorporation.

6.5.2.2 Biochar and compost synergistically promoted R_{SOC} flux

As expected, the addition of compost alone significantly promoted R_{SOC}, likely as a consequence of both microbial growth and stimulation of microbial activity by enhanced C-resources availability (Iovieno et al., 2009). Compost contains a large fraction of labile DOC that can be easily used as substrate by microorganisms. Changes in microbial community composition should also be considered. Furthermore, the improvement of soil physical properties (e.g. SWC) could have contributed to a more favorable environment for microbes (Tejada et al. 2009).

Similar responses to our findings were described by many authors, who concluded that increases of soil respiration and enzyme activities are a direct consequence of organic amendments incorporation in soils (Bastida et al., 2008; García-Gil et al., 2000; Perucci, 1992). CO_2 flux represents a good indicator of the soil OM decomposition rate, indicating that the mineralization process of this substrate proceeded over the monitored period (18 months). Compost decomposition rate is a function of its maturity, soil properties (e.g. C/N ratio) and temperature. Although high values of R_{SOC} often coincides with a higher availability of nutrients for plant (compost mineralization), it indicates a limited potential of such substrates as a C storage strategy.

Noteworthy, the flux of R_{SOC} was synergistically promoted by the combined addition of compost and biochar. The effect was clear since the beginning of the experiment and led to a significantly higher cumulative evolution of CO_2. It should also be mentioned that a significantly greater amount of DOC was often measured in the leachate from biocompost treated soil compared to addition of biochar and compost alone (data not shown). Our evidences do not allow discriminating among C sources emitted both as CO_2 and as fluxed DOC (in the leachate), hence the origin of this additional rate of C remains uncertain. Possible mechanisms include: a) an enhanced biochar-C mineralization or an abiotic release of biochar-C derived induced by the presence of compost; b) an enhanced compost-derived C mineralization stimulated by biochar; c) an increased native SOM mineralization (priming effect) induced by the contemporaneity of the two amendments and, d) all previous mentioned mechanisms occurred at different rates. Although biochar is more stable in soils relative to other sources of OM, it is demonstrated that the

addition of an easily degradable C-rich substrate (i.e. glucose) accelerated its mineralization by several times (Kuzyakov et al., 2009; Hamer et al., 2004). This suggested the so called "co-metabolic" response due to the enhanced growth of microbial biomass and the concurrent increase in enzyme production (Hamer et al., 2004). Increased biochar decomposition could also have occurred when mixed with compost, since the latter acts as an inoculant carrier, providing microorganisms able to promote biochar oxidation and degradation. Nonetheless, the unaltered C concentration of the biochar fragments (biocompost treatment) recovered at the end of the experiment seems to exclude that the additional rate of C is biochar-derived.

On the other hands, improving O_2 availability and providing habitats for microbes (Lehmann et al., 2011), biochar in soil may have stimulated microbial growth and activity which do not depend on biochar-derived C as a source of energy, thereby a further degradation of the compost matrix cannot be excluded. Although the addition of a highly decomposable OM source to a biochar amended soil did not stimulate R_{SOC} rate, it conversely induced an immobilization of the OM by increasing microbial biomass (Steiner et al., 2004).

Another explanation involves possible positives priming effect induced by biochar at the expense of the native soil OM or added easily degradable OM sources (such as compost). Evidences suggest that biochar in soil may promote priming effects, increasing the decomposition of resident soil OM (Kuzyakov et al., 2009; Wardle et al., 2008; Hamer et al., 2004) in particular in sandy soils, as in our case, where native organic C is scarcely protected by clay particles (Fang et al., 2015). However, since biochar alone did not increase R_{SOC}, we believe that a further decomposition of compost could represent the C source of the priming effect.

6.5.2.3 Soil rhizosphere-derived respiration R_R

We were able to estimate rhizosphere-derived respiration (R_R) rates from the second growing season. This because in the first season, values of R_{TOT} and R_{SOC} were always similar, since the contribution of R_R remained negligible likely due to the fact that tree roots were poorly developed and did not colonize yet the soil volume under the R_{TOT} collars. In our conditions and independently of the strategies, R_{SOC} represented most of the R_{TOT}, although R_R accounted, in average,

from 11 to 46% of the R_{TOT}. This indicates that R_R has notable importance in the soil C cycle dynamic. In addition, although poorly correlated (R^2=0.39), the relative contribution of R_R to R_{TOT} was less pronounced in summer. This was mainly attributed to a significantly reduced R_{SOC} flux recorded in winter (soil temperature dependence), rather than a promoted R_R activity in summer.

An increased belowground net primary productivity (NPP) after biochar application has been also indicated as a possible source for an increased CO_2 emission from soil in the long-term (Major et al., 2010). This did not occur in our experiment (data not shown), suggesting that C-related metabolic processes involving root respiration were unaffected by the amendments in the first 18 months growing. Literature reports scarce information about the effect of organic amendments on soil respiration partitioning on fruit tree ecosystems, in particular from biochar (and similarly for biochar mixed with compost) amended soils. Ventura et al. (2014) reported a significantly larger R_{SOC} in biochar-treated soil, especially during summers over 2-year monitoring. The same authors estimated significantly less rhizosphere-derived respiration rates in biochar-treated soil in a mature apple orchard, postulating an effect of biochar on microbial species composition or enhancement of metabolic activity. A better understanding of the impact of biochar, compost and biocompost on soil C fluxes partitioning in cultivated lands may provide evidences to predict the effect of such strategies on soil C and nutrient dynamics.

6.5.3 Soil temperature and temperature dependence

The soil application of either biochar or compost may reduce the surface albedo (amount of solar radiation reflected back in space; Genesio et al., 2012; Meyer et al., 2012) and as a consequence an increase in soil temperature associated to larger soil heat flux may be expected (Ventura et al., 2012; Vaccari et al., 2011). Since OM decomposition rates are linked to soil temperature, an increase of the latter promotes SOC loss. In our conditions, we assume that the shadow induced by the tree canopy and the anti-hail net, as well as irrigations contributed to reduce the solar radiation at soil level or mitigate temperature fluctuations, thus effects were somehow disturbed. However, R_{SOC} was correlated with soil T, which increased

following an exponential shape (Ventura et al., 2014; Reichstein and Beer, 2008). R_{10} was poorly correlated with soil moisture, indicating that soil temperature has a major impact on soil R_{SOC} emission. However, since soil moisture was controlled (May through September) by irrigations, responses in natural ecosystem could be different.

6.5.4 Biocompost induced microbial community shift

Bacterial community was significantly affected by sampling time and soil amendment. Broadly speaking, the cluster analyses showed that the most remarkable shifts were ascribed to the addition of compost and occurred from the first sampling (6 months after amendments incorporation). This occurred mainly since compost is a substrate rich in microbes, containing up to $2x10^9$ colony forming units (CFU) of aerobic bacteria per gram dry matter (Postma et al., 2003), hence once incorporated, compost is an inoculant agent for soil. Furthermore, its simple nature stimulates both the microbial community in the compost substrate itself, as well as the soil-born microbiota community. Conversely, bacterial profiles between unamended and biochar treated soils showed a high similarity at both sampling time. Despite biochar-C derived is considered largely unavailable to microbes (Theis and Rillig, 2009), it is known that the porous structure of biochar may offer micro-habitats for bacteria (Atkinson et al., 2010) and, as a consequence, literature reports significant changes in bacterial soil community upon biochar addition (Chen et al., 2013; Kolton et al., 2011; Hu et al., 2014). Likewise, altering soil physicochemical properties (e.g. porosity, nutrient availability, cation exchange capacity (CEC), WHC and pH), biochar may shift the microbial community structure and function (Ameloot et al., 2014; Anderson et al., 2011; Lehmann et al., 2011, and literature therein). Nevertheless, in agreement with our findings, other studies did not report any effect induced by biochar on bacterial community, (Rutigliano et al., 2014; Dempster et al., 2012), suggesting that eventual shift depends on the type of biochar and environment-conditions.

Although bacterial community in biocompost amended soil share more than 50% similarity with that of the compost, the combination of the two amendments exhibited a unique profile with an increase in band richness. The positive effect of

organic amendments on bacterial community diversity and complexity in soil is well documented (Chaudhry et al., 2012; Vivas et al., 2009; Fracchia et al., 2006). In our study biocompost promoted the bacterial band richness likely as a consequence of the interaction between the two amendments. We hypothesize that the porous structure and the high surface area of biochar provided an aerated habitat in which soil bacteria, inoculated with compost, were able to flourish safer than in the amendments separated. Furthermore, compost provided readily decomposable C sources for soil-born microbiota which developed faster and were physically protected by biochar.

The identification of the most relevant bands revealed the presence of *Arthrobacter* spp., which is a widespread soilborne bacterial genus (Garbeva et al., 2004). However band intensity was clearly higher in unamended and biochar amended soils compared to compost-based treatments suggesting a possible reduction due to the emergence of new microbial population. *Planifilum fimeticola* was mainly detected in amended soils; it belongs to the Thermoactinomycetaceae family, firstly isolated from compost by Hatayama et al. (2005).

Actinomadura flavalba (band 5), *Saccharomonospora viridis* (band 7), *Thermosporomyces composti* (band 8) and *Enterobacter* spp. (band 9 and 10) were peculiar of compost and biocompost soil profiles and, except for *Enterobacter* spp., these microbes belong to the compost microbial consortia (Xu et al., 2013); nevertheless, the presence of *Enterobacter* spp. have been found during the later stages of composting of sewage solids (Novinscak et al., 2009) and in mature manure compost-amended soil (Edrington et al., 2009), indicating that compost represents a source of biodiversity in soil.

6.5.5 Biocompost promoted gene expression of *Nitrosomonas* spp. and *Nitrobacter* spp. bacterial community

Belonging to the Nitrobacteraceae family, *Nitrosomonas* spp. and *Nitrobacter* spp. are genus of rod-shaped, gram-negative and chemolithotrophic (requiring O) bacteria, able to use inorganic reduced compounds as a source of energy. They both are involved in the N cycle in soil by increasing the availability of N to plants. The first genus oxidizes ammonia (NH_3^+) into nitrite (NO_2^-) as a metabolic process,

while *Nitrobacter* spp. uses energy from the oxidation of NO_2^- into NO_3^-, which is then the N mineral form available for plant uptake. Both genus are typical of cultivated lands, especially in sub-alkaline and rich of N compounds soils (Prescott et al., 2005) in which they prefer to colonize solid and smooth surfaces. Optimum pH for *Nitrosomonas* spp. ranges between 6.0 and 9.0 while *Nitrobacter* spp. prefers between 7.3 and 7.5. *Nitrosomonas* spp. membranes contain AMO, the key enzyme for NH_3^+ oxidation leading to the formation of hydroxylamine first and then to NO_2. The latter will be oxidized by bacteria belonging to the genus *Nitrobacter* spp. through the NOR enzyme. When protein rich OM is incorporated into the soil, the nitrification rate is enhanced.

Amendments significantly promoted expression of key genes (AMO and NOR) involved in nitrification cycle and the corresponding *Nitrosomonas* spp. and *Nitrobacter* spp. relative abundance in soil, supporting the notion that in our study amendment strategies affected the N cycle whit the most significant changes observed in the soil treated with biocompost. Providing an aerated habitat for bacteria and increasing soil CEC and DOC, the mere addition of biochar to soil may stimulate the nitrifying community. In addition, biochar may favor the adsorption of inhibiting nitrification molecules with positive effect on nitrifying bacteria (Ameloot et al., 2013 and literature therein). This was also observed in our experiment, although significant differences between unamended and biochar treated soils were only sporadically measured. This occurred, at least partially, because the soil we used was intentionally sandy and poor of OM, thus available protein-rich substrates for nitrifying bacteria were limited. Such response is also in agreement with the observed trend in the R_{SOC}, which remained similar between the two treatments. On the other hand, soil respiration rates were similar despite a higher microbial reproduction rates induced by glucose addition in soils amended with biochar (Steiner et al., 2004). However, biochar-induced changes were observed mainly in the last soil sampling (Sep-13) for both *Nitrobacter* spp. abundance and *Nitrosomonas*-induced enzymatic gene expression, suggesting that changes were likely at the beginning and that required more time. Furthermore, these changes do not seem to be related to a liming effect or an improved WHC in the biochar treated soil.

Interestingly, the positive effect induced by the addition of compost was enhanced by the mixture with biochar. This effect was evident since the first sampling (6 months after amendment incorporation into the soil) and proceeded throughout the experiment timecourse. We measured an increased concentration of mineral N in soil in compost-amended soils, suggesting the positive effect induced by this amendment on nitrifying bacteria. However, assuming a similar N uptake by plants (data not shown), an increased availability of soil mineral N (in particular as NO_3^--N) was expected to emerge in the biocompost amended soil. Such availability was not measured, indicating that NO_3^--N likely followed a different pattern (e.g. lost through leaching). In addition, the significantly higher concentration of N recovered on the biochar fragments at the end of the experiment could have contributed to effectively retain part of the NO_3^-- N in the soil amended with biocompost. Recently, in fact, Kammann et al. (2014) suggested a strong role of biochar in retaining mineral N, mostly in the form of NO_3^- rather than NH_4^+. Furthermore, we suppose that part of the N (as NO_3^-- N) was assimilated by the growing microbes in soil, inducing an N immobilization which did not alter N mineral availability.

6.6 CONCLUSIONS

Benefits on soil properties and fertility were mainly induced by the addition of compost (either with or without biochar) on the sandy sub-alkaline soil, endorsing its agronomical value as soil conditioner. Different C sequestration potential emerged between compost and biochar, since the latter showed a greater stability in soil over the first 18 months following its application at a relatively high rate (87 t ha^{-1}), while the increased release of CO_2 from compost was directly linked to the stimulation of the microbial community by providing labile C sources.

However, interacting synergistically, the mixture of the two amendments, significantly affected C cycle in soil promoting R_{SOC} and, despite the source of C emissions were unclear, a priming effect induced by biochar on the labile C-fractions supplied with compost is hypothesized. This response reflects immediate negative ecological implications because of the faster C losses in the environment. However, it may account only for a small fraction of the C totally stored with biocompost, suggesting that the potential to sequester C in soil can even result enhanced compared to the addition of the amendments alone. The synergism between the two strategies lead to a further improvement of the agronomical soil properties (e.g. higher SWC) and this occurred also in the N cycle, through the promotion of bacterial communities involved in the nitrification process (*Nitrosomonas* spp. and *Nitrobacter* spp.). The bacterial community analysis by PCR-DGGE showed that biodiversity was accentuated by compost and, to a greater extent, by biocompost which displayed a unique profile with a significant increase in band richness and bacterial species derived from the compost inoculum. We suggest that the porous structure of biochar provided an aerated habitat for bacteria inoculated with compost which provided easily decomposable C sources for soil-born microbiota. However, the lack of responses in specific bacterial communities observed in biochar amended soil suggests that sometimes benefits from biochar are overestimated. On the other hand, in the period of investigation, hardwood-derived biochar in a sandy soil had no detrimental effect on specific bacterial diversity, rather promoted the bacterial communities involved in the nitrification process. *Actinomadura flavalba*, *Saccharomonospora viridis*, *Thermosporomyces composti* and *Enterobacter* spp. were peculiar of compost and, in particular, of

biocompost profile. We conclude that the contemporaneous addition of biochar and compost in soil appear effective from an agronomical point of view, providing ecosystem services and offering new technology for the sustainable management of natural resources (including organic wastes), although environmental concerns (C emissions, leaching of DOC and N), require further investigations.

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Table 6.1. Physical and chemical characteristics of the soil used in the experiment

Parameter	**Method[1]**	**Unit**	**Value**
Sand (2-0.05 mm)	Bouyoucos	g kg^{-1}	880
Silt (0.05-0.002 mm)	Bouyoucos	g kg^{-1}	90
Clay (<0.002 mm)	Bouyoucos	g kg^{-1}	30
Organic Matter	Walkley-Black	g kg^{-1}	5.5
Total C		g kg^{-1}	3.19
C/N ratio			7.78
pH (in water)			8.07
Total carbonate ($CaCO_3$)	De Astis	g kg^{-1}	190
Active lime ($CaCO_3$)	Drouineau	g kg^{-1}	1.1
Cation Exchange Capacity (CEC)	Barium Chloride	meq 100 g^{-1}	10.65
S.A.R. index			0.26
Chlorotic power index			71
Electrical conductivity		mS cm^{-1}	0.164
Total N	Kjeldhal	g kg^{-1}	0.41
Chloride water soluble		mg kg^{-1}	14
P exchangeable[2]	Olsen	mg kg^{-1}	26
P_2O_5 exchangeable[2]		mg kg^{-1}	60
K exchangeable[3]	Barium Chloride	mg kg^{-1}	87
K_2O exchangeable[3]		mg kg^{-1}	104
K water soluble		mg kg^{-1}	7.4
Ca exchangeable[3]	Barium Chloride	mg kg^{-1}	1914
Ca water soluble		mg kg^{-1}	71.9
Mg exchangeable[3]	Barium Chloride	mg kg^{-1}	79
Ma water soluble		mg kg^{-1}	4.8
Na exchangeable[3]	Barium Chloride	mg kg^{-1}	53
Na water soluble		mg kg^{-1}	8.3
Fe exchangeable[3]	DTPA	mg kg^{-1}	12.4
Mn exchangeable[3]	DTPA	mg kg^{-1}	6.2
Cu exchangeable[3]	DTPA	mg kg^{-1}	1.49
Zn exchangeable[3]	DTPA	mg kg^{-1}	0.76
B exchangeable[2]	hot water	mg kg^{-1}	0.32

[1]Analisys were performed according to National Official Methods (D.M. 13/09/1999 G.U. N, 248 of 21/10/1999).

[2]Determined spectrophotometrically

[3]Determined by AAS (Atomic Absorption Spectrophotometry)

Table 6.2. Physical and chemical characteristics of the biochar

Parameters	**Unit**	**Value**
Physical properties		
Moisture	%[1]	13.8
Bulk density	g cm^{-3}	0.43±0.04
Hydrophobicity		Slightly hydrophobic
Total porosity	mm^3 g^{-1}	2722
Transmission pores	mm^3 g^{-1}	318
Storage pores	mm^3 g^{-1}	1997
Residuals pores	mm^3 g^{-1}	406
Max water absorption	g g^{-1} of d.m.	4.53
Skeletal density (SD)[2]	g cm^{-3}	1.86±0.04
Envelope density (ED)[3]	g cm^{-3}	0.2459±0.0056
Porosity (ED/SD)	%	0.863±0.00574
Surface area[1] (BET Brunauer–Emmett–Teller method)	m^2 g^{-1}	410±6
Particle size distribution[1]	mm g^{-1}	
50-20	%	4.45
20-10	%	12.1
10-8	%	13.1
8-4	%	10.36
4-2	%	19.85
2-1	%	24.2
<1	%	15.94
Chemical properties		
pH	-	9.8
CEC	cmolc kg^{-1}	101
Carbon[1] (C)	g kg^{-1}	778.0
Total nitrogen (N)	g kg^{-1}	9.1
C/N	-	85.49
Aluminum (Al)	mg kg^{-1}	268
Arsenic (As)	mg kg^{-1}	0.005
Beryllium (Be)	mg kg^{-1}	0.001
Cadmium (Cd)	mg kg^{-1}	0.001
Calcium (Ca)	g kg^{-1}	25.0
Chrome (Cr)	mg kg^{-1}	0.002
Cobalt (Co)	mg kg^{-1}	0.002
Copper (Cu)	mg kg^{-1}	97
Iron (Fe)	mg kg^{-1}	333
Magnesium (Mg)	g kg^{-1}	28.7
Manganese (Mn)	mg kg^{-1}	84
Molybdenum (Mo)	mg kg^{-1}	2
Phosphorus (P)	g kg^{-1}	23.3
Potassium (K)	g kg^{-1}	13.9
Sodium (Na)	g kg^{-1}	11.9
Sulphur (S)	mg kg^{-1}	481
Zinc (Zn)	mg kg^{-1}	104

[1]data obtained from Baronti et al. (2014) (with permission). [2]The skeletal density is the sample mass divided by sample volume occupied by a solid sample, including any pores not accessible to the helium gas. [3]The envelope density is defined as the sample mass divided by the total sample volume that is measured if an "envelope" would be placed around each individual particle.

Table 6.3. Main physical and chemical parameters of the compost

Parameters	**Unit**	**Value**
Humidity	%	47.9
pH (in water)		7.5
Specific conductivity	dS cm^{-1}	3.52
Salinity	meq 100 g^{-1}	84.5
Plastic materials < 5 mm	% d.w.	<0.01
Plastic materials > 5 mm	% d.w.	<0.01
Other inerts < 5 mm	% d.w.	<0.01
Other inerts > 5 mm	% d.w.	0.33
Salmonella	[1]MPN g^{-1}	none
E. coli	[2]CFU g^{-1}	<25
Organic matter	g kg^{-1} (d.w.)	543.1
Organic Carbon (C)	g kg^{-1}(d.w.)	386
Humic and Fulvic C	g kg^{-1}(d.w.)	141
Total nitrogen (N)	g kg^{-1}(d.w.)	22.7
Organic N	% of total N	87.2
C/N		17.0
Chrome hexavalent (Cr)	mg kg^{-1}	<0.5
Cadmium (Cd)	mg kg^{-1}	<0.5
Sodium (Na)	mg kg^{-1}	3385.3
Lead (Pb)	mg kg^{-1}	31.1
Copper (Cu)	mg kg^{-1}	87.1
Zinc (Zn)	mg kg^{-1}	189.8
Mercury (Hg)	mg kg^{-1}	<0.5
Nickel (Ni)	mg kg^{-1}	15

[1]most probable number
[2]colony-forming unit
Source: Nuova Geovis, Bologna, Italy, (2012) – Analyses report N. 11.4235

Table 6.4. Effect of soil-applied Biochar and Compost on soil pH

Treatment	**Date**								
	May-12	**Jul-12**	**Sep-12**	**Nov-12**	**Jan-13**	**Mar-13**	**May-13**	**Jul-13**	**Sep-13**
Control	7.81 ab	7.74	7.72 a	7.89 a	7.83 a	7.80 a	7.98 a	8.00 ab	7.93 a
Biochar	7.92 a	7.80	7.78 a	7.86 a	7.82 a	7.91 a	8.05 a	8.06 a	7.96 a
Compost	7.63 b	7.72	7.55 b	7.68 b	7.57 b	7.54 b	7.73 b	7.91 b	7.77 b
Biocompost	7.61 b	7.66	7.56 b	7.64 b	7.52 b	7.61 b	7.72 b	7.90 b	7.85 b
Significance	*	ns	*	**	*	*	***	**	*

ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test)

Table 6.5. Effect of soil-applied Biochar and Compost on soil NO_3^--N and NH_4^+-N concentration (mg kg^{-1})

Treatment	**May-12**		**Jul-12**		**Sep-12**		**Nov-12**		**Jan-13**		**Mar-13**		**May-13**		**Jun-13**		**Jul-13**		**Sep-13**	
	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N
Control	6.6 b	2.9 b	9.6 b	3.7 b	1.1 b	2.3	0.3 b	2.3	<dl	<dl	<dl	<dl	37.6 a	3.1	27.4	2.9	2.5	2.2	0.73 b	0.72 b
Biochar	6.7 b	2.7 b	9.8 b	3.2 b	1.8 b	2.1	1.2 a	2.4	<dl	<dl	<dl	<dl	20.3 a	3.3	40.3	6.1	3.6	2.0	0.84 b	0.80 b
Compost	12.0 a	4.0 a	44.2 a	4.2 a	2.8 a	2.4	0.7 ab	2.7	<dl	0.08 a	<dl	0.39 a	1.6 b	3.2	18.1	3.1	4.0	3.1	1.52 a	1.37 a
Biocompost	12.1 a	4.0 a	50.1 a	4.5 a	3.6 a	2.5	1.8 a	2.8	<dl	0.09 a	<dl	0.45 a	2.4 b	2.7	20.4	4.0	2.0	2.3	1.34 a	1.28 a
Significance	***	**	***	*	*	ns	*	ns	ns	**	ns	*	*	ns	ns	ns	ns	ns	*	**

ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test). dl= detection limit was 0.1 and 0.6 µg L^{-1} for NO_3^- and NH_4^+, respectively.

Table 6.6. C and N concentration of the soil and of the biochar fragments at the end of the experiment

Treatment	**Soil**		**Biochar Fragments**	
	C	**N**	**C**	**N**
	g kg^{-1}		g $100g^{-1}$	
Control (Fresh[1])	2.29 b	0.55 c	77.6	0.23 b
Biochar	2.32 b	0.77 c	73.1	0.66 a
Compost	2.78 b	1.50 b	-	-
Biocompost	4.71 a	2.51 a	73.3	0.50 a
Significance	***	**	ns	***

ns, ** and ***: effect of treatment not significant or significant at P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test).[1] Fresh indicates biochar fragments never applied to the soil.

Table 6.7. Effect of soil-applied Biochar and Compost on soil moisture (0.05-0.30 m) throughout the experiment

Treatment	**Soil moisture (g 100 g^{-1} dw)**									
	May-12	**Jul-12**	**Sep-12**	**Nov-12**	**Jan-13**	**Mar-13**	**May-13**	**Jun-13**	**Jul-13**	**Sep-13**
Control	13.4 b	12.8 b	3.2 b	11.7 b	14.4 c	12.9 c	9.7	5.2 b	6.1	7.8 b
Biochar	17.4 a	14.5 b	3.4 b	13.3 ab	16.6 bc	14.2 b	10.7	6.4 ab	6.6	8.8 ab
Compost	17.2 a	15.6 ab	4.0 b	14.2 ab	17.2 ab	15.4 b	11.9	7.5 ab	6.9	10.1 a
Biocompost	22.5 a	18.8 a	5.3 a	15.5 a	19.4 a	17.4 a	11.9	8.5 a	7.2	10.3 a
Significance	*	**	*	**	***	***	ns	*	ns	**

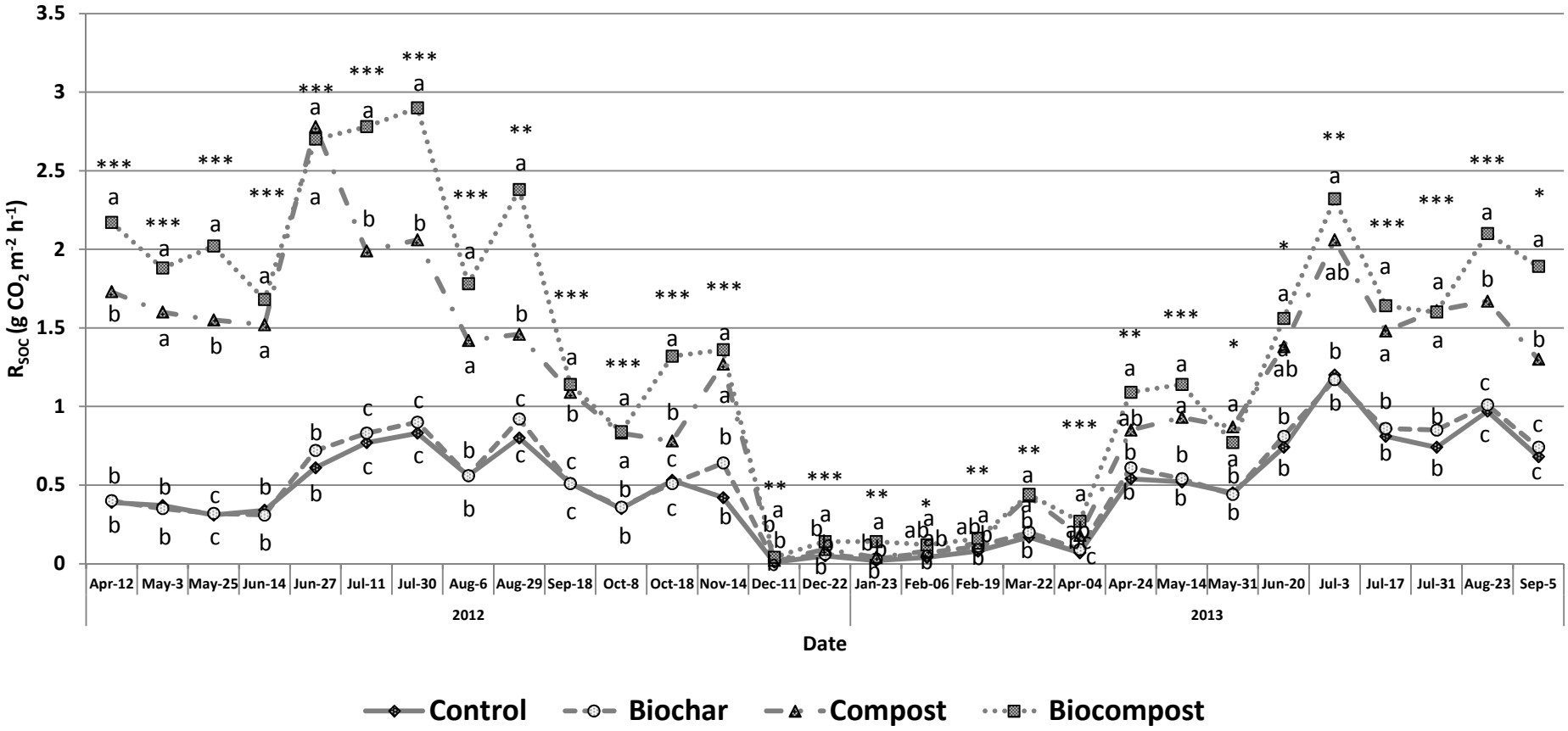
ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. In the same column, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test)

Table 6.8. Phylogenetic identification by BLAST alignment tool (http://www.ncbi.nlm.nih.gov/BLAST/) of selected DGGE bands from the bacterial DGGE fingerprint as shown in figure 6.9

Band number[a]	**Closest Identity**	**% Identity**[b]
1	*Planifilum fimeticola*	99%
2	nd[c]	
3	*Gloeobacter kilaueensis*	94%
4	nd	-
5	*Actinomadura flavalba*	100%
6	*Arthrobacter spp.*	100%
7	*Saccharomonospora viridis*	100%
8	*Thermasporomyces composti*	97%
9	*Enterbacter spp.*	100%
10	*Enterbacter spp.*	100%

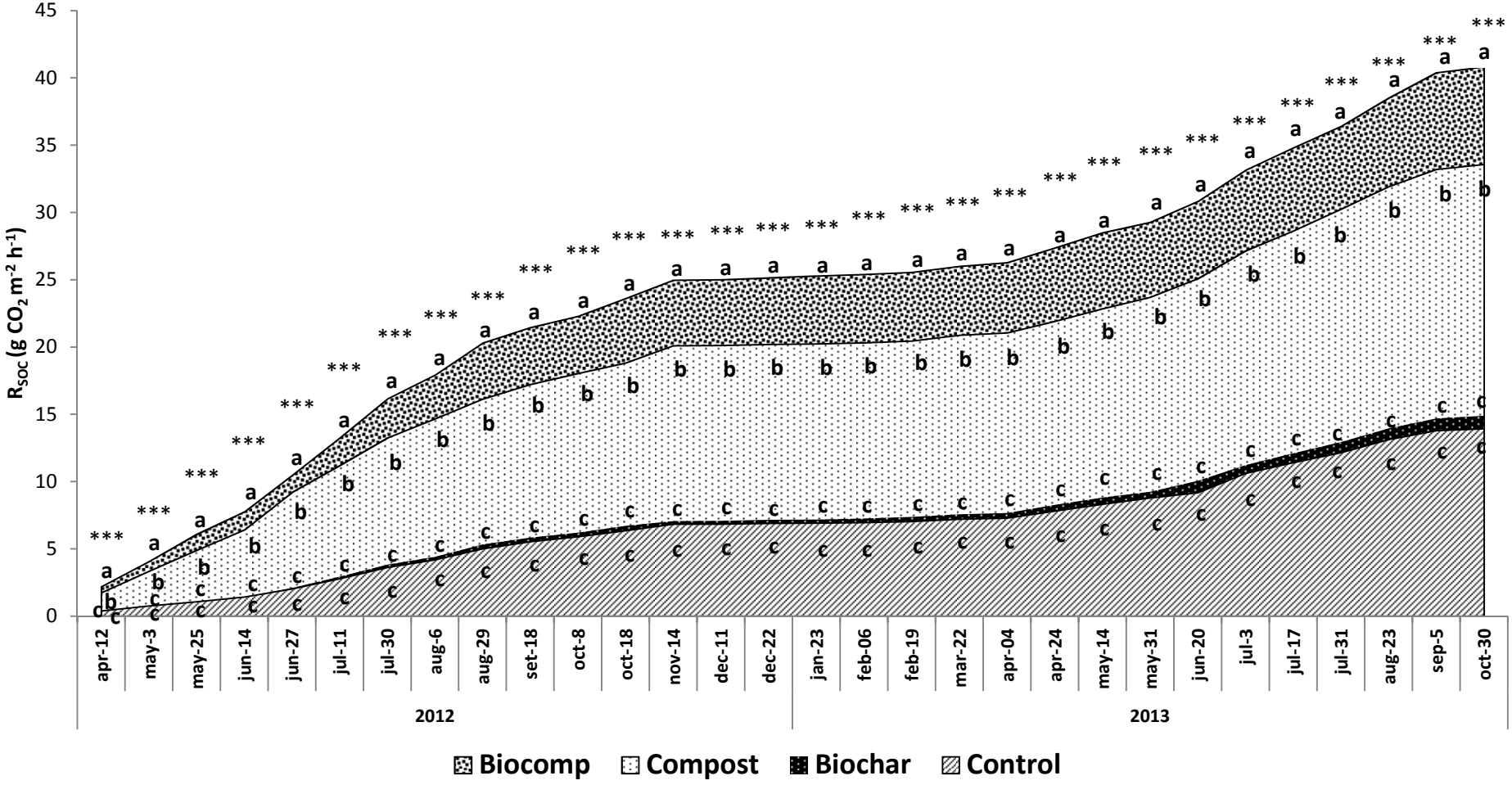
[a]Bands are numbered according to Fig. 9.
[b]Identity represents the % identity shared with the sequences in the GenBank databases assignment of band sequences from PCR-DGGE profiles.
[c]not determined

Figure 6.1. Effect of soil-applied biochar and compost on soil organic C-derived respiration (R_{SOC})



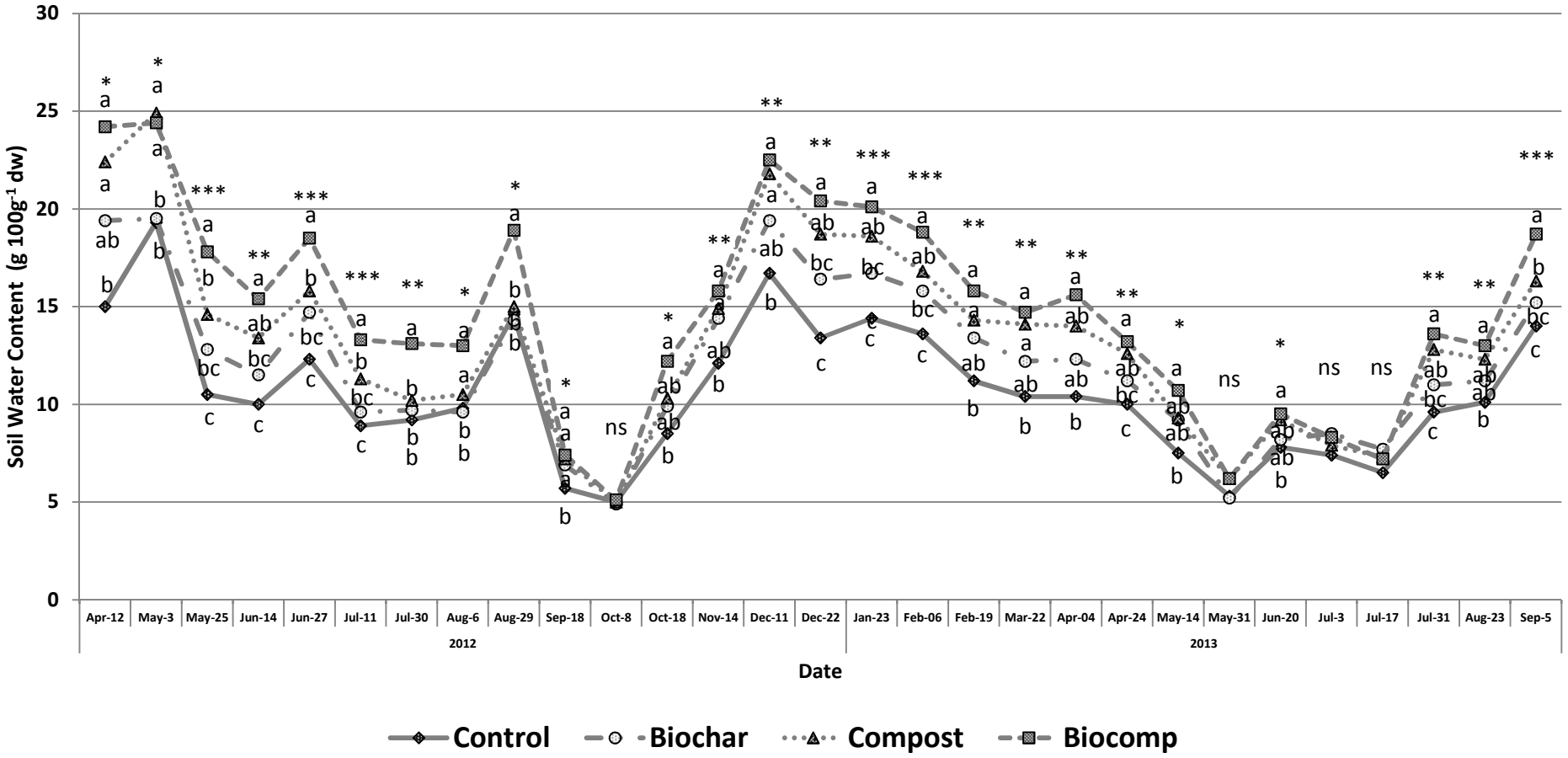
*, ** and ***: effect of treatment significant at P≤0.05, P≤0.01 and P≤0.001, respectively. Within the same date, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test). Values represent means of 4 replicates.

Figure 6.2. Cumulative evolution of soil organic C-derived (CO_2) respiration (R_{SOC}) as affected by soil-applied biochar and compost



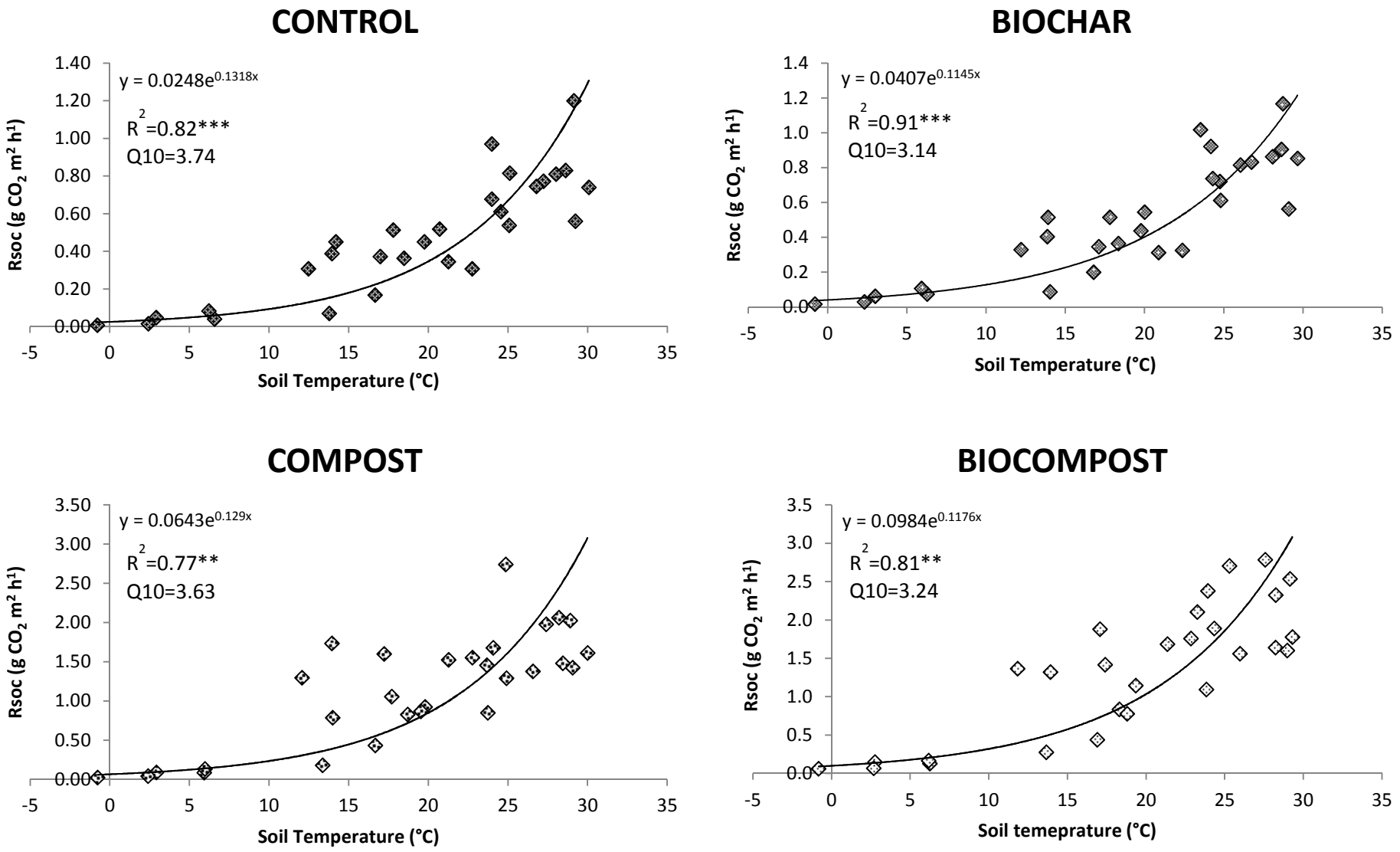
***: effect of treatment significant at P≤0.001. Within the same date, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test). Values represent means of 4 replicates.

Figure 6.3. Effect of soil-applied biochar and compost on soil water content (SWC) at 0-0.05 m depth



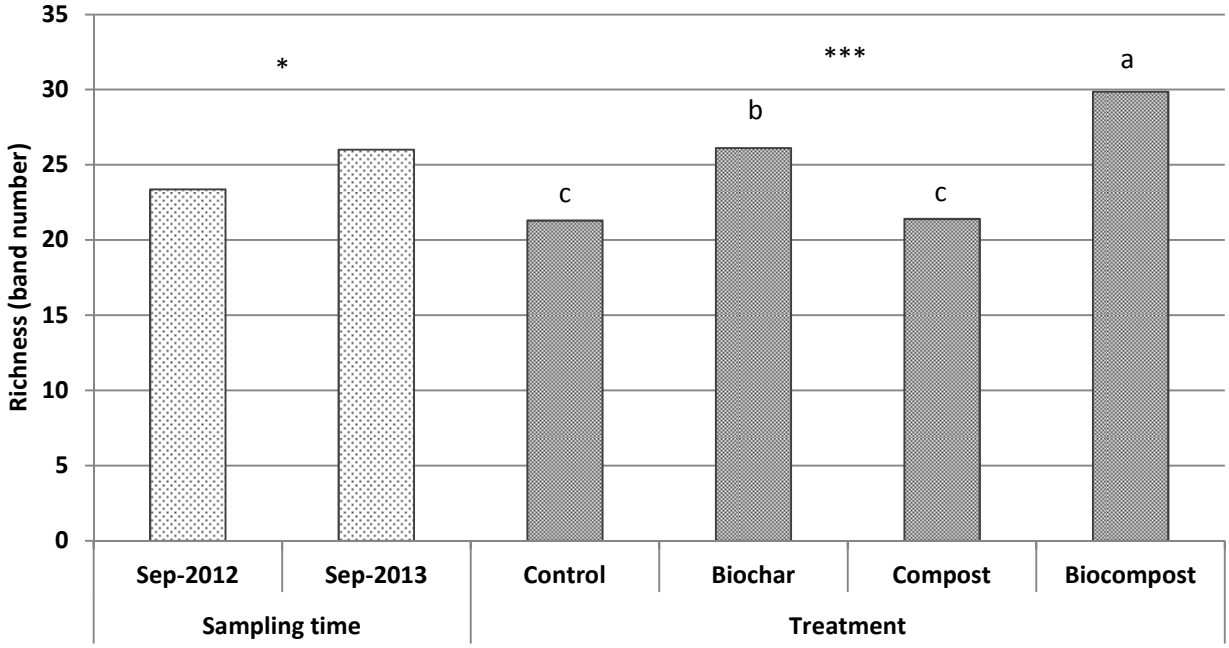
ns, *, ** and ***: effect of treatment not significant or significant at P≤0.05, P≤0.01 and P≤0.001, respectively. Within the same date, means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test). Values represent means of 4 replicates.

Figure 6.4. Effect of soil-applied biochar and compost on temperature dependence of soil organic C-derived (CO_2) respiration (R_{SOC})



** and ***: correlation between soil temperature and R_{SOC} significant at P≤0.01 and P≤0.001, respectively.

Figure 6.5. Genetic richness of the bacterial community in soil unamended and amended with biochar, compost and biocompost sampled after 6 (Sep-12) and 18 months (Sep-13) amendment application



* and ***: effect of sampling time and treatment significant at P≤0.05 and P≤0.001, respectively. Within the same factor (sampling time and treatment), means followed by the same letter are not statistically different (P≤0.05, Tukey's HSD Test). Interaction between sampling time and treatment not significant.

Figure 6.6. Clustering analysis of the DGGE patterns of the rhizospheric soil analyzed at two sampling times (Sep-2012 and Sep-2013). CTR, B, C and BC indicate soil samples unamended and amended with biochar, compost and biocompost, respectively

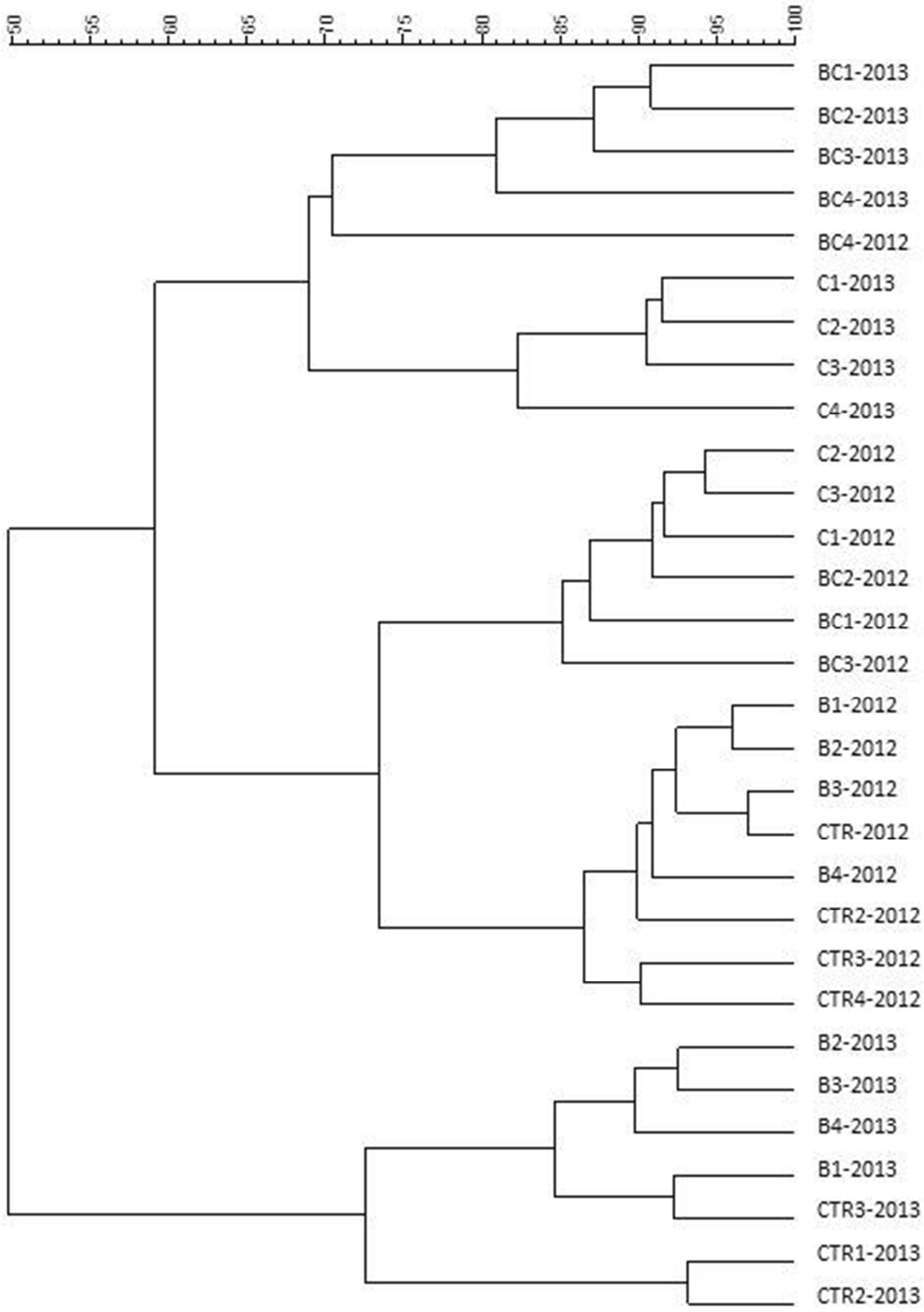


Figure 6.7. Multidimensional scaling (MDS) analysis of DGGE profiles (16S rRNA gene) from rhizobacterial communities analyzed at two sampling times (Sep-2012 and Sep-2013)

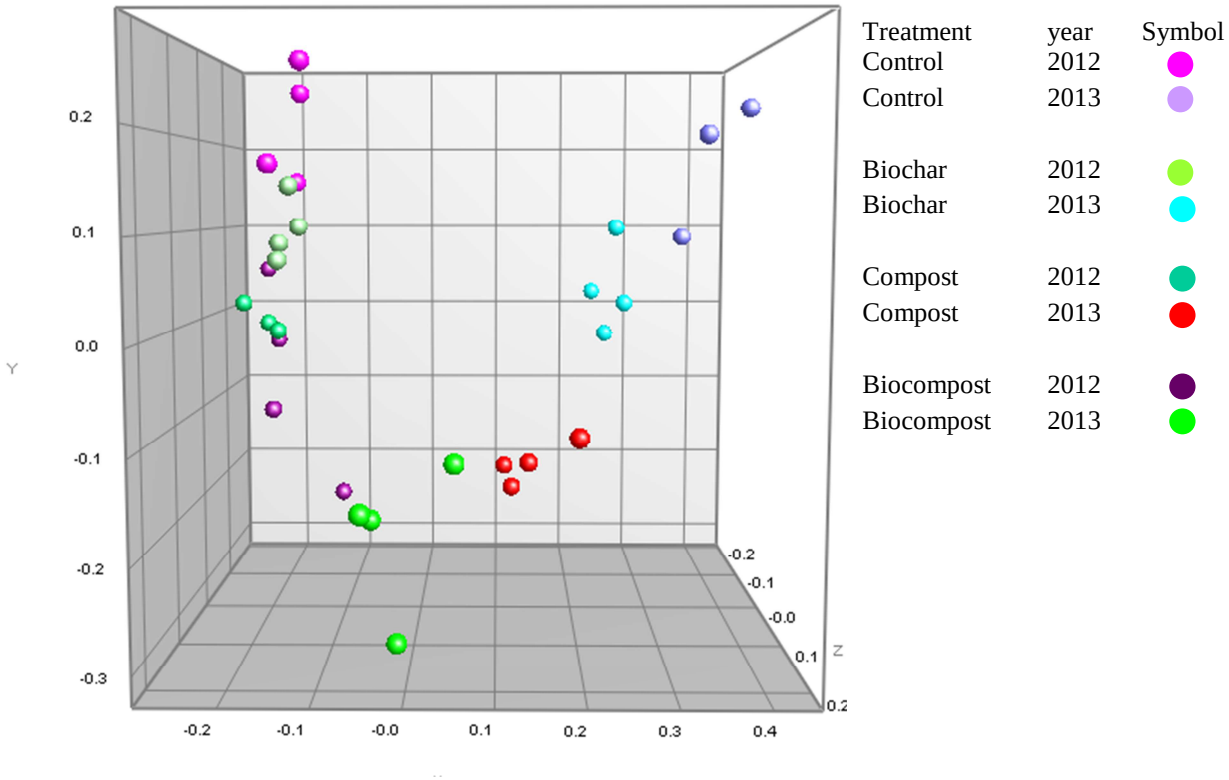


Figure 6.8. Principal Component Analysis (PCA) applied to bacterial diversity in the soil at two sampling times (Sep-2012 and Sep-2013). CTR, B, C and BC indicate soil samples unamended and amended with biochar, compost and biocompost, respectively. The axis 1 and 2 explain the 20.4% and 37.2% of total variance, respectively,

Ctr 2012; ♦ Ctr 2013; B 2012; ■ B 2013; ◊ C 2012; ★ C 2013; ○ BC 2012; ● BC 2013

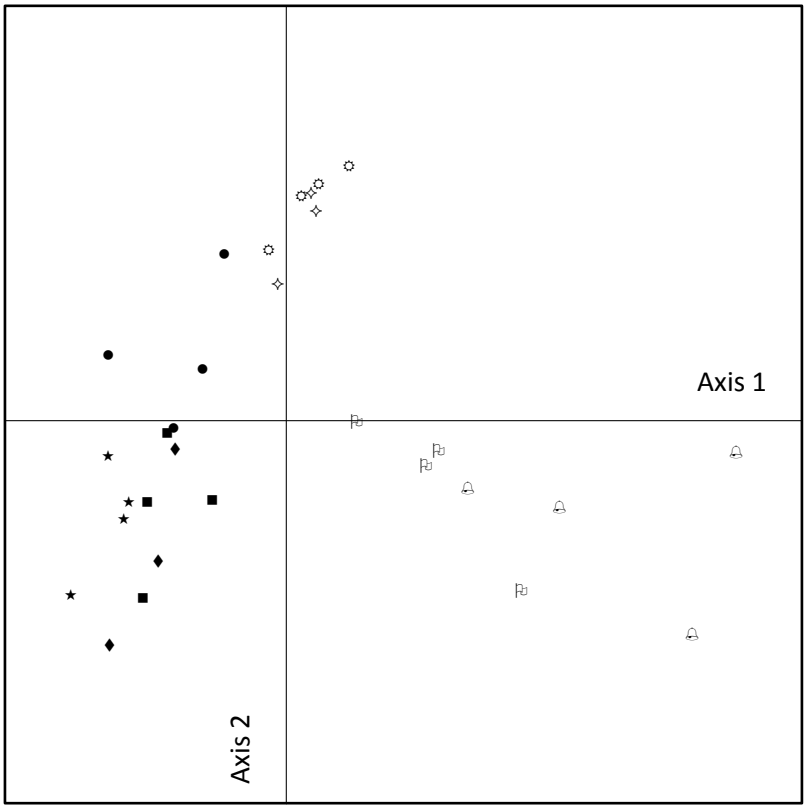


Figure 6.9. DGGE patterns of 16S rDNA fragments amplified from rhizospheric soil at the end of the experiment (Sep-2013). Arrows indicated the most relevant bands excised

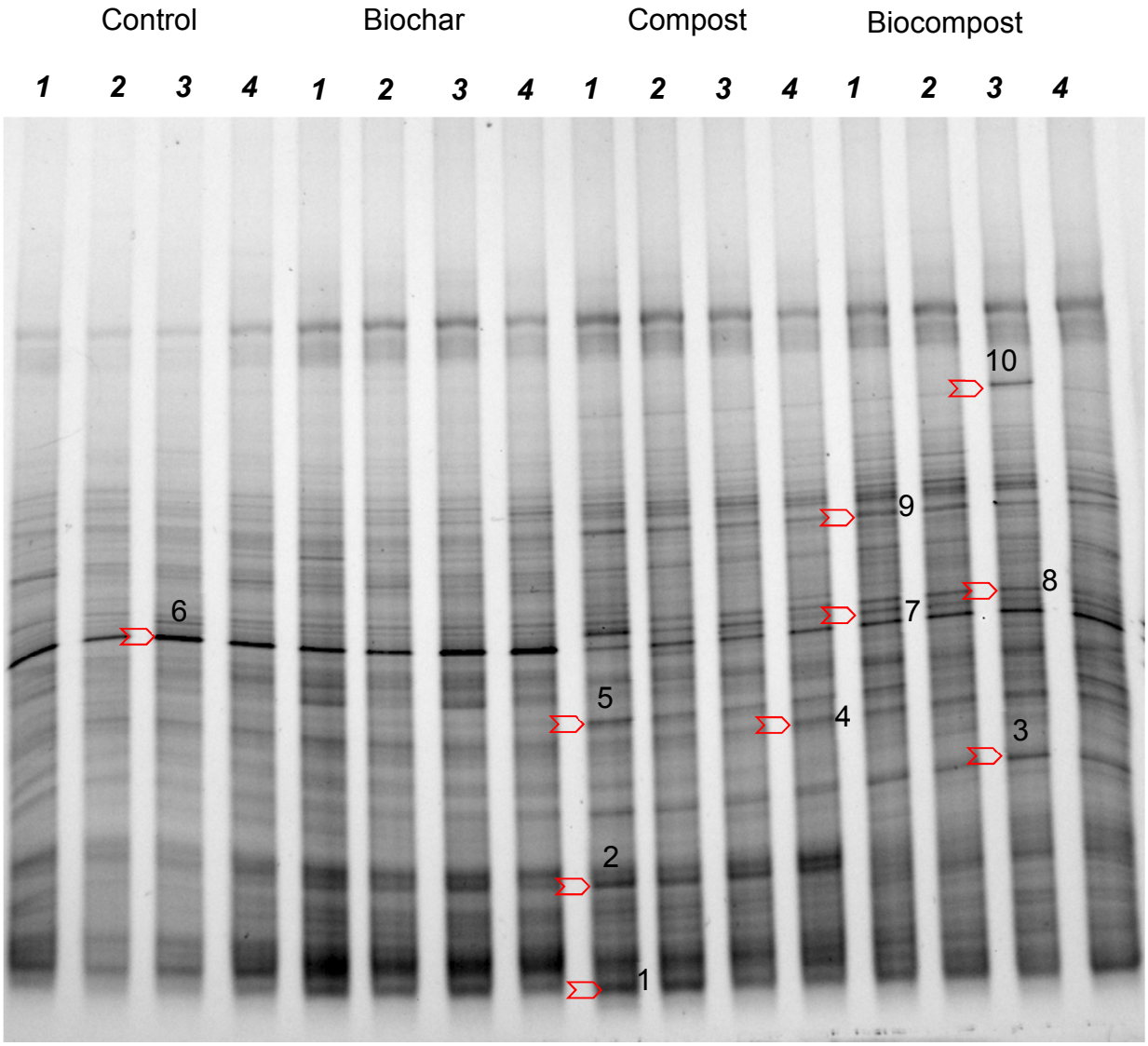
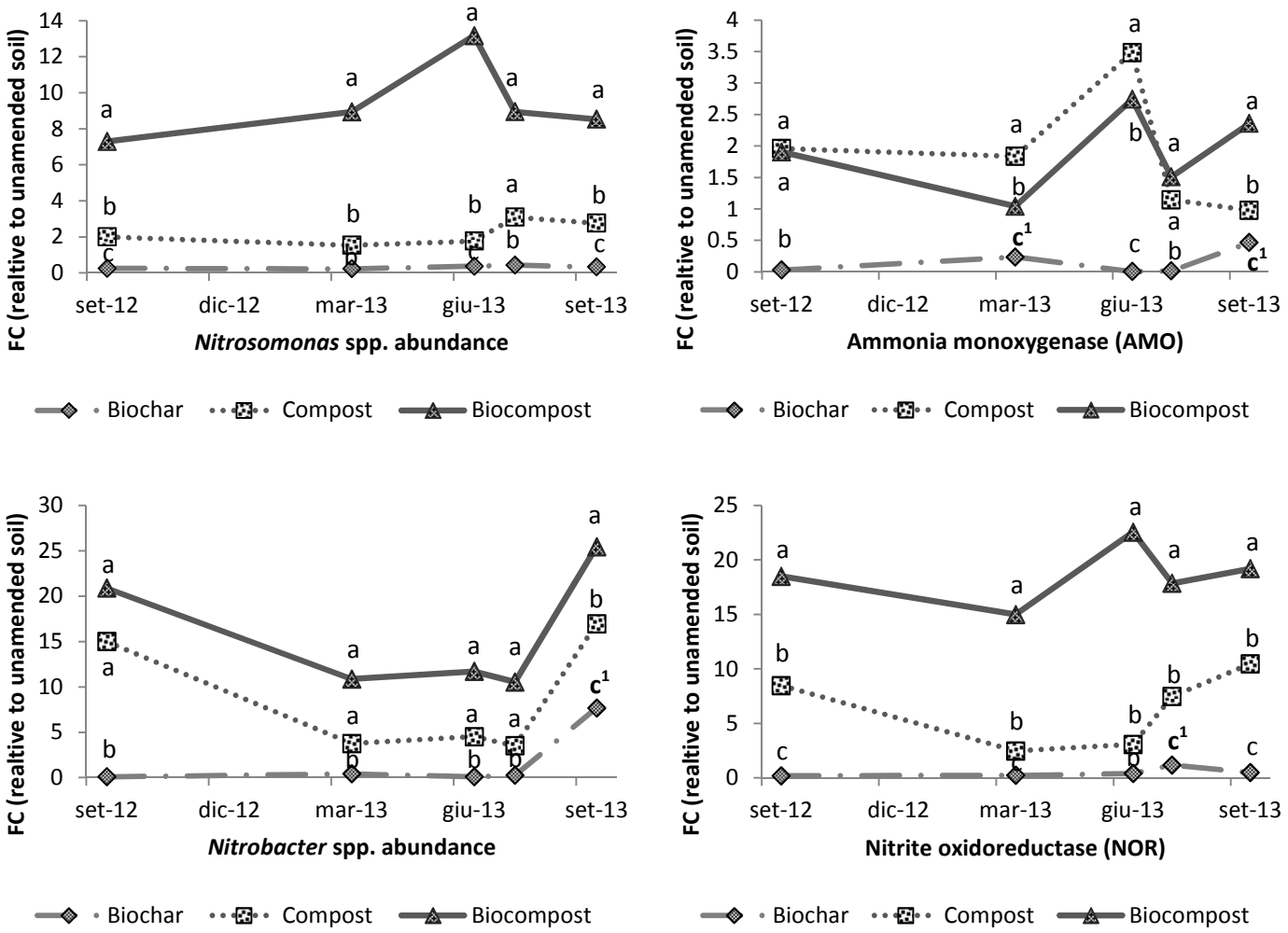


Figure 6.10. Effect of soil-applied biochar and compost on gene expression of key genes of nitrification by *Nitrosomonas* spp. (Ammonia monoxygnease) and *Nitrobacter* spp. (Nitrite oxidoreductase) species and relative bacterial abundance in soil



Data are expressed as time fold changes (FC) expression levels relative to reference genes expression level. For each sampling, different letters indicate statistical difference according to the SNK test ($P \leq 0.05$). Compost and biocompost were always significantly different from unamended soil, whereas $\mathbf{c^1}$ indicate a statistical difference between biochar and control.

CHAPTER 7

Biochar on nutrient retention and crop performance in temperate region: a 3-year field trial in a nectarine orchard

Abstract

Biochar has the potential to alter soil water holding capacity and macronutrients availability (either releasing or retaining minerals), thus benefit plant growth and crop yield. Nevertheless, long-term field-trials in temperate regions are limited and benefits from biochar might not always be as evident as for highly weathered tropical soils. Therefore, the aims of this study were to: i) evaluate the potential of biochar as a source of macronutrients and its affinity in retaining these; ii) assess the effect of increasing biochar rates on soil properties and mineral N retention under natural leaching conditions; iii) investigate the long-term effect of biochar rates on nutritional status, yield and fruit quality of nectarine trees grown in temperate region. To this end, a set of lab tests showed that biochar released sustained amounts of phosphorus (P), magnesium (Mg) and, in particular, potassium (K). Biochar released low amounts of ammonium nitrogen (NH_4^+-N) while that of nitrate-N (NO_3^--N) was absent. Moreover, biochar was ineffective in removing most of the cations (and NO_3^--N) from enriched solutions, while at the rate of 40 g L^{-1}, biochar removed almost 52% of the initial NH_4^+-N concentration (10 mL^{-1}).

A 3-year field trial was carried out in a mature, irrigated, fertilized, commercial nectarine orchard (Big Top grafted on GF677) grown on a sandy-loam soil located in the Po Valley, where increasing biochar (from hardwood pyrolyzed at 500°C) application rates (0, 5, 15 and 30 t ha^{-1}) were compared. Soil pH, soil moisture, soil mineral N availability and leaching, leaf nutritional status, yield and fruit quality were evaluated. Results from the field showed that biochar did not affect soil quality and plant response over three years of investigation. We hypothesize that, unless an evident constraint is identified, in non-limiting conditions in terms of water availability and soil fertility, potential benefits from biochar application for

plant nutrition and soil fertility are hidden or negligible. It is worth noting that biochar was not harmful to either nectarine trees or soil properties, indicating that rates can be increased or application repeated. However, independently of the rates, biochar reduced the leaching of NH_4^+-N, (but not that of NO_3^--N), whit both environmental and agronomical advantages.

Keywords: Nitrogen, macronutrients, Big Top, soil leaching, ion exchange lysimeters

7.1 INTRODUCTION

Biochar is the recalcitrant carbon (C)-rich byproduct of the thermal decomposition of biomasses under limited oxygen supply at relatively low temperatures (pyrolysis or gasification) (Atkinson et al., 2010). Biochar as a soil conditioner is progressively gaining interest as a valuable strategy to increase C sequestration and mitigate climate change (Laird, 08), providing at the same time benefits for both soil fertility and crop yield (Atkinson et al., 2010).

An overall increase in crop productivity has been reported following biochar application (Genesio et al., 2015; Jeffery et al., 2011). Nevertheless, yield response to biochar addition is not always positive, but can be neutral (Schmidt et al., 2014) or even negative (Mukherjee and Lal, 2014; Crane-Droesch et al., 2013; Spokas et al., 2012; Van Zwieten et al., 2010 and literature therein), suggesting that that crop response to biochar application varies with crop species, environmental conditions, soil type, biochar characteristics (feedstock and charring conditions) and application rate. Most of the scientific evidences on the use of biochar as a soil conditioner were obtained in tropical and subtropical environments, where soils are highly weathered with low soil organic C (SOC) and cation exchange capacity (CEC) (Glaser and Birk, 2012). Moreover, experiments were often short-term and carried out in controlled conditions (with limited environmental fluctuations) and results are frequently contradictory (Mukherjee and Lal, 2014). Few studies focused on the effect of biochar on perennial crops grown in field conditions in temperate region (Hammond et al., 2013; Jeffery et al., 2011), likely due to the longer time required to produce detectable effects on species with a largely developed root system (Genesio et al., 2015). Consequently, performing long-term evaluations on the response of different crops in field conditions grown in diverse environments has been stressed as a research priority (Lorenz and Lal, 2014; Mukherjee and Lal, 2014), as biochar-induced effects on soil properties and crop response may also change over time (Lentz and Ippolito, 2012).

Rates at which biochar should be applied in different conditions to achieve positive responses, or at least to avoid detrimental effects, is also still uncertain. Glaser et al. (2001) suggest that moderate biochar application rates are usually beneficial despite in few cases were negative, at least for some crops or soils (Gaskin et al.,

2010; Van Zwieten, et al., 2010; Sohi et al., 2009). Identify mechanisms behind observed yield responses also in relation to the application rate is therefore of crucial importance (Mukherjee and Lal, 2014; Spokas et al., 2012).

To explain how biochar might benefit plant growth and crop yield, different mechanisms have been proposed, including the supply of nutrients, the improvement of nutrient use efficiency, and the alteration of soil chemical-physical parameters (e.g. pH and bulk density) that affect plant growth, soil water retention and plant available water (Baronti et al., 2014; Mukherjee and Lal, 2014; Atkinson et al., 2010; Sohi et al., 2010; 2009; Lehmann and Joseph, 2009). Biochar can act as a fertilizer or amendment, by either supplying or retaining minerals in soil (Laird et al., 2010; Silber et al., 2010; McHenry, 2009; Steiner et al., 2008; 2007; Lehmann et al., 2003). Content, form and chemical structure of minerals in biochar are strongly influenced by the pyrolysis conditions and biomass. Usually, during thermal degradation, potassium (K), chlorine (Cl) and nitrogen (N) vaporize at temperatures below 700 °C, while calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S) vaporize at higher temperatures (>1000°C) (Amonette and Joseph, 2009). These elements concentrate in the biochar as the progressive elimination of the more volatile C, oxygen (O) and hydrogen (H) occurs (Singh et al., 2010; DeLuca et al., 2009; Gaskin et al., 2008). From an agronomical point of view, rate and extent at which nutrients contained in biochar become available for plant uptake and how biochar interacts with minerals dissolved in the soil solution is of major importance to guide fertilization.

Biochar can increase soil nutrient retention because of its ability to absorb ions, due to its high surface area and charge density (Lehmann, 2007; Liang et al., 2006). Several studies confirm the ability of biochar to retain both nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) decreasing N losses through leaching (Kamman et al., 2014; Ventura et al., 2013; Lehmann et al., 2003). However, evidences about the effect of biochar in retaining other nutrients, as well as the rate at which it is more effective in adsorbing inorganic N in agricultural soils under leaching conditions are still scarce, in particular in intensive cultivated lands (Ding et al., 2010).

Therefore, the aims of this study were to: i) evaluate the potential of biochar as a source of macronutrients and its affinity in retaining these; ii) assess the effect of

increasing biochar rates on mineral N retention under natural leaching conditions; iii) investigate the long-term effect of biochar rates on yield, nutritional status and fruit quality in a perennial crop grown in temperate region;

Results could provide knowledge to best guide the exploitation of the biochar approach. To address these aims, were performed a set of lab tests to evaluate macronutrients releasing and retention capacity of biochar. In addition, a 3-year field experiment was carried out in a commercial nectarine orchard

We hypothesized that: i) biochar can represent a source of macronutrients and increase nutrient retention in soil; ii) benefits from biochar to soil implies positive response on yield, nutritional status and fruit quality and iii) increasing application rates can proportionate increasing benefits.

7.2 MATERIALS AND METHODS

7.2.1 Biochar macronutrients release and retention potential

The biochar used in this experiment was provided by Romagna Carbone (Bagnacavallo, RA, Italy) and obtained in a transportable ring kiln where a mixed feedstock of mechanically chipped hardwood (mainly from peach and grapevine) was slowly pyrolyzed at approximately 500 °C, at atmospheric pressure. A complete physicochemical characterization of the biochar has been performed is reported in table 7.1. Biochar was sieved at 2 mm mesh (fragment size ranged between 2 and 7.5 mm), then repeatedly washed in deionized water (d-H_2O) (20:1 w w^{-1}) by shaking for 30 min at 100 rpm on an orbital shaker to reduce ash and tar content. Electrical conductivity (EC) of the solution was determined by a conductimeter (Conductivity meter 524, Crison, Barcelona, Spain), and washing procedure was repeated (7 times) until EC reached the constant value of 51.5 ± 1.64 µS (the initial EC was 661 ± 8.83 µS, n=3). Washed biochar was then oven-dried at 30°C for 48 h and then added to 100 mL glass flasks (Pic. 7.1) containing 25 mL of d-H_2O in order to obtain the final biochar concentration of 4, 10, 20, 30 and 40 g L^{-1} with six replicates for each concentration. Additionally, 2 series of flasks with same rates of biochar were added with 25 mL solutions containing 10 mg L^{-1} of either NO_3^--N or NH_4^+-N. Pure KNO_3 (99.7 % purity, Merck) or $SO_4(NH_4)_2$ (99 % purity, J.T. Baker) salts were used as a source of NO_3^--N or NH_4^+-N, respectively. The pH of each solution was adjusted to 7.2 ± 0.1 with 1 M sodium hydroxide (NaOH) or 0.1 M HCl and then flasks were shaken 120 min at 100 rpm on an orbital shaker. The supernatant was filtered (Wathmann 42) and finally analyzed for NO_3^--N and NH_4^+-N concentration with a continuous flow autoanalyser (AA-3; Bran+Luebbe, Norderstadt, Germany).

Picture 7.1. Biochar samples were weighted at increasing rates

Additionally, 5 series of flasks, with same rates of biochar as previously indicated with 3 replicates, were added with 25 mL of either d-H_2O or solutions containing 10 mg L^{-1} of one of the following cations: K, P, Mg or Ca. Pure K_2O diluted in HNO_3, P_2O_5 diluted in d-H_2O, $MgCl_2 \times 6H_2O$ and $CaCl \times 6H_2O$ diluted in HCl 0.12 N standard solutions (Sigma-Aldrich) were used as a source of macronutrients. The pH of all the solutions was adjusted by 1 M NaOH or 0.1 M HCl at 7.2 ± 0.1, and then flasks were shaken on an orbital shaker for 120 min at 100 rpm. The supernatant was filtrated (Wathmann 42) and analyzed for macronutrients by AAS (Varian AA200, Mulgrave, Victoria, Australia).

7.2.2 Field trial

7.2.2.1 Plant material and growth conditions

A 3-year (2009-12) field trial was carried out on a mature commercial nectarine (*Prunus persica* (L.), Batsch) orchard of the cv. Big Top grafted on the hybrid GF677 (*P. persica* L. x *P. amygdalus* L.) planted in 1997 with a frame of 3.5 m × 5.5 m (519 trees ha^{-1}). The orchard was located in the South Eastern Po Valley (Tebano, Ravenna, Italy, 44° 29' N, 11° 78'E, 58 m a.s.l.) on a sandy-loam soil classified as Inceptisol (USDA, 2010), which main physical and chemical characteristics are summarized in table 7.2. Climate of the area is classified as temperate sub-continental with cold winters and humid and warm summers. Throughout the experiment, the average air temperature was 13.6 °C, while annual precipitation ranged between 650 and 790 mm, mainly concentrated in spring and autumn. Trees were trained as in a delayed vase and drip irrigated from May to August in order to return the evapotranspiration rate. Orchard alleys were maintained with native grass species and tree rows (2 m wide) herbicided with glufosinate ammonium (DL-phosphinothricin), twice per year. Trees were yearly thinned and managed in terms of pruning, irrigation as well as pest and disease control, according to the regional guidelines of Integrated Crop Management (ICM, 2009). Fertilization was managed by providing 0.25 kg N $tree^{-1}$ (130 kg N ha^{-1}) as Urea (46% N) distributed yearly at petal fall.

7.2.2.2 Experimental design

The biochar used in this trial was taken from the same batch used for the lab assay. Four biochar application rates (0, 5, 15 and 30 Mg ha^{-1}) were compared in a complete randomized block design (Pic. 7.2), with 5 replicates arranged in 4 consecutive tree rows. Each experimental plot consisted of 5 trees, and only the 3 central trees were used for data collection. Adjacent plots along the row were separated by at least 2 unamended trees. In November 2009, biochar was distributed on the herbicided strip (2 m wide) along the tree row of each experimental plot (35 m^2 area) and incorporated into the soil at the depth of 20 cm (A horizon) by a disk arrow. The same soil disturbance was applied on unamended plots.

Picture 7.2. Plot amended with biochar, before its incorporation

7.2.2.3 Leaf chlorophyll content and tree nutritional status

From July 2010, leaf chlorophyll (Chl) content was estimated in summer on 10 fully expanded leaves per tree, randomly selected from the annual shoots, using a hand-held Chl meter (SPAD 502, Minolta Co. LTD, Osaka, Japan). The same leaves were then collected, immediately closed into polyethylene bags and transported in a portable refrigerator to the laboratory, where leaf area was determined with a LI 3000 leaf area meter (Li-Cor Inc., Lincoln, Nebraska, USA). Leaf laminas (without petiole) were washed in a 0.1 N HCl solution with 0.1% surfactant (Tween 20, Sigma-Aldrich, Milan, Italy), rinsed 2 times in tap water, then in d-H_2O, oven dried (65 °C) and milled (0.2 mm mesh). Specific leaf weight (SLW) was calculated dividing leaf dry weight by leaf area. Leaf N and P concentration was determined by Kjeldahl method (Schuman et al., 1973) and spectrophotometric quantification at 700 nm (Saunders and Williams, 1955), respectively. Leaf metals (K, Ca, Mg, iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu)) were extracted by wet mineralization according to US EPA Method 3052 (Kingston, 1988) and determined by atomic absorption spectrophotometry

(AAS) (Varian AA200, Mulgrave, Victoria, Australia), as described in Sorrenti et al. (2012).

7.2.2.4 Tree yield and fruit quality

At commercial harvest in 2011 and 2012, tree yield was determined and fruit weight and quality were evaluated on a subsample of 26 healthy fruits per plot (Pic. 7.3).

Picture 7.3. At commercial harvest, yield and fruit weight were determined

Fruit firmness was measured individually on two opposite faces of peeled fruits by a hand pressure tester FT 011, (EffeGi, Ravenna, Italy) fitted with an 8 mm diameter plunger. Fruit soluble solids content (SSC) was determined on the fruit juice by a digital refractometer (Digital Refractometer PR-1, Atago, Tokio, Japan), while 20 mL of juice were added to 20 mL of d-H_2O and titrated with 0.1 N NaOH to the endpoint of pH 8.1 for titratable acidity (TA) (expressed as malic acid) and juice pH determination, using a Compact Tritator I (Crison, Barcelona, Spain). Only in 2012, fruit flesh samples were lyophilized, milled and used to determine mineral concentration (N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn) following the procedures used for leaves as previously described.

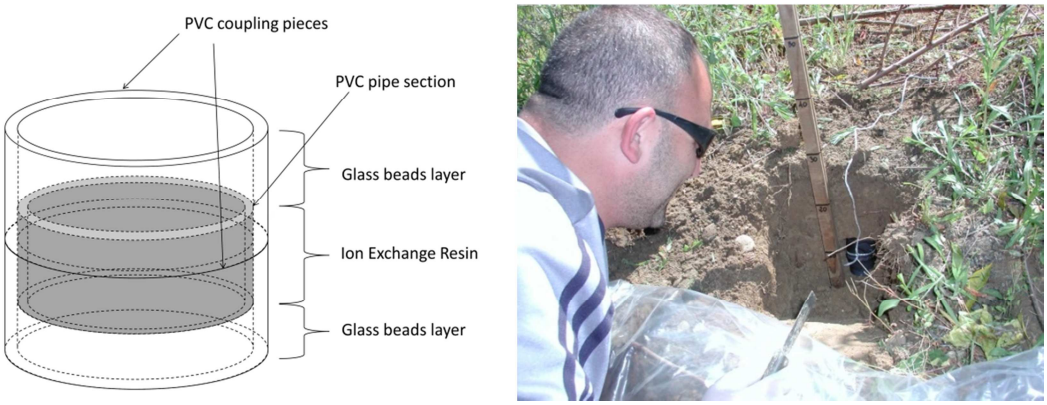
7.2.2.5 Soil analysis

Soil pH was determined on soil cores collected (5-30 cm depth) every 6 months. Samples were oven dried (105°C) and ground (2 mm mesh), then 10 g were added to 25 mL of d-H_2O shaken for 1 h at 95 rpm on an orbital shaker. The pH was measured on the filtered supernatant with a pH-meter (BasiC 20, Crison, Barcelona, Spain) under continuous stirring.

Soil cores were collected three times (spring, summer and autumn) per year at a depth of 5-30 cm. Cores were, ground (2 mm mesh) and extracted by shaking in 2 M KCl solution (1:10 ww^{-1}) for 1 h at 95 rpm on an orbital shaker. Extracts were filtered (Whatman 42) and analyzed for NO_3^--N and NH_4^+-N concentration with a continuous flow autoanalyser (AA-3; Bran+Luebbe, Norderstedt, Germany). Soil moisture content (w w^{-1}) was evaluated gravimetrically by oven drying at 105 °C representative subsamples of the same cores.

7.2.2.6 Mineral N leaching

To assess the effect of the biochar rates on the mineral N leaching, ion-exchange resin lysimeters were assembled as described by Susfalk and Johnson (2002) and adapted by Ventura et al. (2013). Briefly, 20 g of a mixed ion-exchange resin (Amberlite® MB-150 Mixed Bed Exchanger, gel form, 16-50 mesh) were trapped in polyvinyl chloride pipe (Pic. 7.4) sections (46.4 mm internal diameter) by 2 nylon 125-μm meshes (Scubla s.n.c., Remanzacco, UD, Italy). Washed sand was placed at the two extreme ends of each lysimiter to prevent the contact of the resin with soil and nylon mesh occlusion. Four ion-exchange resin lysimeters per treatment (1 per plot) were buried at 25 cm depth on May 5, 2011 between two adjacent trees, approximately 30 cm aside from the tree row line. Lysimeters were placed vertically and the above soil layer was carefully kept undisturbed (Pic. 7.4). On June 12, 2012, lysimeters were recovered from the soil and mineral N was extracted by washing the resin with a 2 M KCl solution at a ratio of 1:10 (w w^{-1}).



Picture 7.4. Schematic representation (left) and positioning (right) of the ion exchange resin lysimeters

Samples were shaken for 2 h at 95 rpm on an orbital shaker, the supernatant was filtered (Whatman 42) and then analyzed for NO_3^--N and NH_4^+-N concentration as previously described for soils extracts. Background NO_3^--N and NH_4^+-N content of the washed sand used in the lysimeters was measured using the same extraction procedure and subtracted from the amounts recovered in the resins. Recovery capacity coefficients of 84 % and 88 % for NO_3^--N and NH_4^+-N, respectively, were applied to calculate the total amount of mineral-N leached through the lysimiters (Ventura et al., 2013).

7.3 STATISTICAL ANALYSES

Coefficient of determination (R^2) between biochar rate and macronutrients concentration was calculated for the lab assay using linear regression analysis. Data of the field experiment were submitted to analysis of variance (ANOVA) according to a complete randomized factorial design with 2 factors: biochar rate (4 levels) and year (3 levels) with 5 replicates. Data collected for one season were analyzed as in a complete randomized design. When analysis of variance showed statistical effect (at $P \leq 0.05$), means were separated by Student-Newman-Keuls Test (SNK); when interaction between biochar rate and year was significant, 2 times standard error of means (SEM) was used as the minimum difference between two statistically different means (Saville and Rowarth, 2008). Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC).

7.4 RESULTS

7.4.1 Biochar macronutrients release and retention potential

Water-extractable NO_3^--N released by biochar was negligible and unaffected by rate (Fig. 7.1). Similarly, increasing rates of biochar did not affect the initial NO_3^--N concentration in washing solution (Fig. 7.1). Conversely, a small but significant increase of NH_4^+-N concentration was observed in d-H_2O as a consequence of biochar application, linearly related with application rates (Fig. 7.1), indicating a small but significant release of NH_4^+-N from biochar. However, when biochar was added to the 10 mg L^{-1} NH_4^+-N solution, the initial N concentration decreased, according to the application rate (Fig. 7.1). At the highest application rate (40 g L^{-1}), biochar removed 51.7% of the NH_4^+-N initially present in the solution.

The amounts of K, P, and Mg released by biochar in d-H_2O linearly increased with application rates (Fig. 7.2). Biochar released mainly K, followed by P and Mg (Fig. 7.2). The relation between biochar rate and Ca release was best described by a polynomial function of a 2nd degree (Fig. 7.2) and its concentration in d-H_2O increased until the biochar rate of 20 g L^{-1}, then decreased at higher rates.

Once biochar was dipped in 10 mg L^{-1} solutions of K, P, Ca or Mg (separately) the concentration increased for all macronutrients and values where always above 10 mg L^{-1} (data not shown). K, P and Mg concentration was linearly correlated with biochar rates and R^2 were 0.94, 0.92 and 0.37, respectively (data not shown). Ca concentration was significantly increased by the presence of biochar in the 10 mg L^{-1} Ca solution, and the trend was described by a polynomial function of a 2nd degree (data not shown).

7.4.2 Agronomic performance of nectarine trees and soil parameters

Unless for Mn, values of leaf Chl and nutrient concentration showed significant seasonal variations (Tab. 7.3). Leaf Chl and Zn concentration significantly decreased along the three years, while Ca, Mg and Cu showed the lowest values in the first season (Tab. 7.3). Leaf K and Mg were higher in 2011, while leaf Fe concentration was significantly higher in 2012 than in the previous years (Tab. 7.3).With the exception of Fe, no interaction between biochar rates and year was

observed on leaf Chl, leaf macronutrient concentration (Tab. 7.3), leaf area and SLW (data not shown).

Among macronutrients, biochar rates affected only leaf Mg concentration, which was significantly higher in untreated trees compared with those amended with 15 t ha^{-1} biochar. Leaf Mn, Cu and Zn concentration were not affected by biochar rate while leaf Fe concentration was decreased in biochar amended trees in 2011 and 2012, irrespective of the application rate, except for the 15 t ha^{-1} in the last year (Tab. 7.3). Yield, fruit weight, SSC, titratable acidity, juice pH and fruit flesh mineral concentration were unaffected by treatments and season (data not shown). On average, productivity was 26.2 kg $tree^{-1}$ while fruit weight was 128 g $fruit^{-1}$. Only fruit firmness resulted significantly higher when biochar was applied at 5 and 15 t ha^{-1} (data not shown).

Biochar rate and year did not alter soil pH, NO_3^--N, NH_4^+-N availability and soil moisture along investigation (data not shown).

7.4.3 Biochar effect on mineral N losses

The amount of NH_4^+-N leached over the 13 months through ion-exchange lysimiters was higher than that of NO_3^--N. Biochar significantly reduced the cumulative amount of NH_4^+-N leached with a similar extent among application rates (Fig. 7.1) while no differences were recorded on NO_3^--N losses. However, the NH_4^+-N leaching reduction due to biochar treatments was on average less than 1.5 kg ha^{-1}.

7.5 DISCUSSION

7.5.1 Biochar as a source of macronutrients

Biochar may provide conspicuous amount of nutrients to plants (Atkinson et al., 2010; Silber et al., 2010) and positive responses of crop yield have been associated to the direct addition of available plant nutrients such as P, K, Ca, Zn and Cu (Alburquerque et al., 2013; Lehmann and Rondon, 2006). However, total mineral content in biochars may not necessarily reflect its potential to supply those nutrients to plants (Spokas et al., 2012). Our tests revealed that our biochar did not release NO_3^--N and released low amounts of water soluble NH_4^+-N. On the contrary, the release of P, Mg and in particular K was considerable, and linearly correlated whit rate, whereas a polynomial trend of a 2nd degree was found for Ca. This response because N in biochar is bound in the recalcitrant organic molecules thus not readily soluble and available for plant uptake (Chan and Zhihong, 2009), whilst the other cations are largely converted into inorganic forms and conserved into biochar (Angst and Sohi, 2013; Amonette and Joseph 2009).

In agreement with previous studies (Gaskin et al., 2010; Silber et al., 2010; Yao et al., 2010) we found a relatively high releasing of K in solution. This is related to the high solubility of K salts contained in biochar which can be rapidly released in soil in available form to plants, often within the first year following application (Angst and Sohi, 2013). Based on our lab results and assuming the highest biochar application rate of our field trial (30 t ha^{-1}), we estimate that 27.5, 2.5 and 1.1 kg ha^{-1} of K, P and Mg, respectively, were potentially supplied to the soil upon biochar incorporation. Although these amounts cannot fulfill plant requirements, it should be mentioned that the biochar used in our lab tests was repeatedly washed prior extraction to reduce ash and tar content, thus we suppose that most of the amount of ready-soluble salts were removed, therefore we expect that a much larger amount of such minerals would have been released from biochar. However, our estimations are in line whit findings of Angst and Sohi (2013) who estimated that, assuming an application rate of 20 t ha^{-1}, the amount of K and Mg supplied in the topsoil with maple or sycamore biochar ranged between 20 and 50 kg ha^{-1} and between 0.60-3.34 kg ha^{-1}, respectively. Nevertheless, even assuming that larger

amounts of available P, K and Mg were supplied from our biochar, we did not observe plant responses in field conditions.

Our data do not show evidence of biochar retention potential for K, P and Mg in solution, since when it was dipped in 10 mg L^{-1} of such ion solutions, the final concentration always increased, indicating a net release from biochar. The concomitant occurrence of Ca release and retention is likely responsible for the polynomial trend observed for this cation, which cannot be excluded also for the other cations.

7.5.2 Biochar did not benefit tree responses and soil properties

Three years of monitoring showed no major significant effects induced by increasing rates of biochar on plant nutritional status, yield and fruit quality. Little information is available on the effect of soil-applied biochar on temperate fruit trees species (Blackwell et al., 2009). Recently, Genesio et al. (2015) reported a significant long-term (4 years) increase in grapevine yield following biochar application (22 t ha^{-1}), without affecting grape quality. Schmidt et al. (2014) found only and mostly non-significant effects of biochar application on soil properties, yield and quality of grape upon the application of biochar either alone (8 ton ha^{-1}) or in combination with compost. Similar results were found by Ventura et al. (2013), who applied the same biochar used in the present study in an apple orchard, without observing significant effects, except for Zn, on leaf Chl and nutrients concentrations. However, in that study, the applied rate (10 t ha^{-1}) was relatively low compared to the rates commonly used in field trials (Biederman and Harpole, 2013; Jeffery et al., 2011) while in the present study we decided to increase rates up to 30 t ha^{-1}. Possible explanations to the lack of responses following biochar treatments in our orchard should not be associated with the biochar application rate, but may be due to the specific site conditions. It should be considered that, when biochar treatments were applied, nectarine trees were mature (12-year old), hence their root system was completely developed. Considering the medium-high vigor of the adopted rootstock (GF677), we can suppose that most of the roots extended below the A horizon where biochar was incorporated, thus limiting the potential benefits of the amendment for plants. However, as suggested by Major et al.

(2010), positive effects following by biochar addition in the upcoming years cannot be excluded, once finer biochar fragments move downwards into deeper soil horizons.

Short-term biochar benefits observed in weathered, acidic soils (even at comparable rates than in our experiment) have often been associated with the biochar liming effect and the resulting increase in soil pH after application (Atkinson et al., 2010; Jeffery et al., 2011). We did not measure change in soil pH, despite the high pH of the biochar (pH 9.8) used. This can be attributed to the soil of the orchard that was already alkaline (pH 8.1) and likely buffered by carbonates, thereby very high biochar application rates would be required to affect significantly soil pH. However, the absence of changes in soil pH after three years upon the incorporation of an alkaline biochar in our orchard represents a positive fact from an agronomical point of view. High soil pH hinders the availability of micronutrients (i.e. Fe, Mn, Zn) with negative implications on plant uptake, and a further pH increase in alkaline-calcareous soils would be undesired. Unlikely, the biochar-induced liming effect will appear after several years from its application because the development of carboxylic acids functional groups, as a consequence of weathering processes occurring on the exposed biochar fragments, will lead to a decrease in the concentration of basic sites on the biochar surface (Yao et al., 2010; Cheng and Lehmann, 2009).

Many authors have observed an increase in soil water content after biochar application to soil and this has been addicted for the yield improvement observed after biochar application. For instance, Baronti et al. (2014) applied 22 t ha^{-1} of biochar for two consecutive seasons to a non-irrigated vineyard and reported an increase in soil water content, a reduction of plant water stress and an increase of photosynthetic activity during drought. In the same experimental site, mentioned improvements were responsible for the beneficial effects recorded on grape yield (Genesio et al., 2015).

In our orchard, biochar rates did not alter soil water content, in agreement with results of Ventura et al. (2012), who applied the same biochar on a clay-loamy soil up to 60 t ha^{-1}. In our site, biochar did not affect soil water because annual rainfall are mainly concentrated in spring and autumn, while tree water requirements were

satisfied in summer by the irrigation system and soil moisture was constantly kept close to the field capacity. IN this conditions, photosynthetic activity and physiological processed mediated by water in plant were never constrained or limited by stomata closure.

Based on this evidences, we assume that in non-limiting conditions (such as in our orchard) in terms of water availability, natural soil fertility and external source of minerals (i.e. fertilizers), agronomical benefits from biochar were hidden or negligible. The natural fertility of the soil of our orchard in terms of availability of exchangeable cations (K^+, Ca^{++} and Mg^+) and cation exchange capacity (CEC), may have limited the effect of biochar as a source of nutrients or as a nutrient retention-additive. N tree requirements, for instance, were yearly fulfilled by chemical fertilizer inputs. The external supply of N to the soil could have also reduced the impairment of N availability in soil due to N immobilization by the microbial biomass which may occur after the incorporation of freshly-produced biochar (Sohi et al., 2010). Unlikely, a temporary N deficiency (N immobilization) occurred in our experiment as confirmed by the fact that soil NO_3^--N and NH_4^+-N availability was not affected by biochar application and because leaf N concentration and crop levels were always sustained and similar to the seasons previous the trial establishment. Benefits from biochar in soil could be maximized on weathered and degraded soils, with low CEC, low soil organic C, low pH and relatively non-reactive clay mineralogy (Crane-Droesch et al., 2013). For these reasons, biochar application in temperate regions was frequently found to have scarcely pronounced or even negative effects on soil properties and crop response (Schmidt et al., 2014; Kloss et al., 2014; Biederman and Harpole, 2013; Jones et al., 2012; Jeffery et al., 2011).

7.5.3 Biochar reduced NH_4^+-N leaching

In field conditions, biochar confirmed its ability to reduce NH_4^+-N losses by retaining this ion in the top soil layer. Similar results have been reported by previous studies carried out mainly in controlled environments (soil columns, pots or lysimeters) (Yao et al., 2012; Ding et al., 2010; Laird et al., 2010; Novak et al., 2010; Lehmann et al., 2003), whereas few evidences were obtained in field

conditions (Major et al., 2010). Likewise, the ability of biochar to remove dissolved NH_4^+-N (but not NO_3^--N) was confirmed also in the lab assay of this study (Fig. 7.1). The effect of biochar in retaining cations has been attributed to its high surface area and to the presence of both polar and non-polar surface sites (Baldock and Smernik, 2002; Cheng et al., 2008), which makes biochar able to sorb (and desorb) cations through electrostatic forces (Alling et al., 2014; Liang et al., 2006). The CEC of biochar typically ranges between 30 and 150 cmolc kg^{-1}, consistently higher than that of clay minerals or OM in soils and permits to firmly bond cations through electrostatic forces. The soil CEC was found to increase over time as a result of the abiotically oxidation and the adsorption of organic matter on the biochar surfaces (Cheng et al., 2006; Liang et al., 2006) resulting on the formation of carboxylic functional groups. As a consequence, the positive charged exchange sites on biochar surfaces decline and negative charge sites may develop as biochar ages (after few months), increasing its ability to retain cations over time (Clough and Crondon, 2010). In our conditions, ion-exchange lysimiters were positioned after almost 2 years from trial establishment and removed after 13 months, thus we suppose that our biochar was highly oxidized and likely it did not affect the NO_3^--N leaching because its anion exchange capacity (AEC) was very low therefore, its potential adsorption for anions was negligible (Hale et al., 2013; Yao et al., 2012; Braker and Conrad, 2011). In contrast to our findings, recent evidences suggest a stronger capacity of biochar in retaining NO_3^--N rather than NH_4^+-N (Kammann et al., 2014). The application of 15 and 30 t ha^{-1} of biochar in a sandy soil significantly increased NO_3^--N concentration in the top soil (0-15 cm) while it was decreased in the deeper layers (up to 90 cm) (Kammann et al., 2014). Similarly, Ventura et al. (2013) found that NO_3^--N leaching was significantly reduced in an apple orchard amended with 10 t ha^{-1} of the same biochar used in the present study. Such divergent results, suggest that biochar potential to retard NO_3^--N and NH_4^+-N losses is affected by different factors other than biochar characteristics and ageing, such as soil properties and ion concentration.

It has been reported that the weak binding between biochar and cations allows a dynamic sorption and release equilibrium, which might increase NH_4^+-N availability for plant roots (Alling et al., 2014). In our study, the reduced leached

amount of NH_4^+-N by biochar addition did not lead to an increase in the availability of NH_4^+-N in soil neither in N plant uptake. This likely because the retained NH_4^+-N amount in amended plots was overall less than 2 kg per hectare saved in more than one year, which represent approximately 1% of the yearly N orchard requirement. Furthermore, biochar reduced the leaching of NH_4^+-N with a similar extent among rates. This could be related to the low availability of NH_4^+-N in the soil or to the fact that increased rates of biochar could have increasingly stimulated microbial biomass and activity (Ameloot et al., 2013) immobilizing increasing rates of N at higher biochar rates.

7.6 CONCLUSIONS

Results of this study confirm that biochar may represent a direct source of plant nutrients (i.e. K, P and Mg) and retain mineral N, mainly under NH_4^+-N form. The latter response was also confirmed in field conditions, which makes biochar a useful strategy to reduce N losses through leaching in agro-ecosystems. However, during three years following its application and up to 30 t ha^{-1}, this potential did not result beneficial to the tree nutritional status, yield, fruit quality or soil properties. The lack of evident benefits may be ascribed to the good soil fertility and water availability of the orchard together with the fact that no specific adverse conditions were recognized in our experimental site before biochar application. Advantages from biochar application are likely to emerge in presence of main constraints for plant growth, such as water stress, toxicity, nutrient deficiencies due to excessive leaching. Therefore, in fertile agricultural soils, under optimal water and fertilizer availability, agronomic benefits from biochar are probably limited.

Nevertheless, biochar was neither harmful to nectarine trees nor detrimental to soil properties during the experimental timecourse, suggesting that application rates can be increased or repeated. In addition, the main purpose of using biochar in conditions similar to our orchard is to increase C sequestration in soil.

7.7 REFERENCES

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Table 7.1. Biochar physical and chemical characteristics

Parameter	Unit	Value
Physical properties		
Moisture	%	13.8
Bulk density	g cm^{-3}	0.43±0.04
Hydrophobicity		Slightly hydrophobic
Total porosity	mm^3 g^{-1}	2722
Transmission pores	mm^3 g^{-1}	318
Storage pores	mm^3 g^{-1}	1997
Residuals pores	mm^3 g^{-1}	406
Max water absorption	g g^{-1} of d.m.	4.53
Skeletal density (SD)[2]	g cm^{-3}	1.86±0.04
Envelope density (ED)[3]	g cm^{-3}	0.2459±0.0056
Porosity (ED/SD)	%	0.863±0.006
Surface area[1] (BET Brunauer–Emmett–Teller method)	m^2 g^{-1}	410±6
Particle size distribution[1]	mm g^{-1}	
50-20	%	4.45
20-10	%	12.1
10-8	%	13.1
8-4	%	10.36
4-2	%	19.85
2-1	%	24.2
<1	%	15.94
Chemical properties		
pH	-	9.8
CEC	cmolc kg^{-1}	101
Carbon[1] (C)	g kg^{-1}	778.0
Total nitrogen (N)	g kg^{-1}	9.1
C/N	-	85.49
Aluminum (Al)	mg kg^{-1}	268
Arsenic (As)	mg kg^{-1}	0.005
Beryllium (Be)	mg kg^{-1}	0.001
Cadmium (Cd)	mg kg^{-1}	0.001
Calcium (Ca)	g kg^{-1}	25.0
Chrome (Cr)	mg kg^{-1}	0.002
Cobalt (Co)	mg kg^{-1}	0.002
Copper (Cu)	mg kg^{-1}	97
Iron (Fe)	mg kg^{-1}	333
Magnesium (Mg)	g kg^{-1}	28.7
Manganese (Mn)	mg kg^{-1}	84
Molybdenum (Mo)	mg kg^{-1}	2
Phosphorus (P)	g kg^{-1}	23.3
Potassium (K)	g kg^{-1}	13.9
Sodium (Na)	g kg^{-1}	11.9
Sulphur (S)	mg kg^{-1}	481
Zinc (Zn)	mg kg^{-1}	104

[1]data obtained from Baronti et al. (2014) (with permission). [2]The skeletal density is the sample mass divided by sample volume occupied by a solid sample, including any pores not accessible to the helium gas. [3]The envelope density is the sample mass divided by the total sample volume that is measured if an "envelope" would be placed around each individual particle.

Table 7.2. Chemical and physical properties of the field soil profile (0-50 cm) at the beginning of the experiment

Parameter	**Unit**	**Value**	**Extractant/method**
Texture			
Sand	%	55	Bouyoucos
Lime	%	33	Bouyoucos
Clay	%	12	Bouyoucos
Total carbonate ($CaCO_3$)	%	12	HCl / De Astis method
Active lime ($CaCO_3$)	%	2.5	Ammonium oxalate (Drouineau, 1942)
Organic matter	%	1.06	Walkley-Black 1919 (Soltner, 1988)
Total N	‰	0.80	Kjeldahl method
Assimilable phosphorus (P)	mg kg^{-1}	8	Olsen (Olsen and Sommers, 1982)
Exchangeable potassium (K)	mg kg^{-1}	97	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable sodium (Na)	mg kg^{-1}	37	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable calcium (Ca)	mg kg^{-1}	2347	Barium chloride (Hendershot and Duquette, 1986)
Exchangeable magnesium (Mg)	mg kg^{-1}	109	Barium chloride (Hendershot and Duquette, 1986)
C/N ratio		7.69	
Cation Exchange capacity (CEC)	meq $100g^{-1}$	13.02	Barium chloride (Hendershot and Duquette, 1986)
pH		8.08	Water/Potentiometric

Table 7.3. Effect of increasing soil-applied biochar rates on leaf Chl content, macro and micro nutrients concentration during 3 years of experiment

Biochar rate (t ha^{-1})	**Leaf Chl Content (Spad Units)**	**Macronutrients (g kg^{-1})**					**Micronutrients (mg kg^{-1})**					
		N	**P**	**K**	**Ca**	**Mg**	**Fe**			**Mn**	**Cu**	**Zn**
							2010	***2011***	***2012***			
0	37.8	20.5	2.31	21.0	34.8	5.71 a	44.2	47.6	63.7	27.9	6.17	36.0
5	37.8	19.5	2.26	21.4	30.6	5.53 ab	43.5	43.8	56.4	28.0	6.11	34.9
15	37.4	20.2	2.34	22.0	31.0	5.42 b	45.4	41.1	62.9	27.2	6.12	36.3
30	37.5	20.0	2.23	21.8	35.0	5.64 ab	45.8	40.4	58.5	27.3	6.01	33.9
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*	*2SEM=3.34*			*ns*	*ns*	*ns*
Year												
2010	39.4 a	21.4 a	2.42 a	22.7 b	28.5 b	4.93 c				27.1	5.65 b	42.7 a
2011	37.5 b	18.4 c	2.16 b	26.7 a	36.3 a	6.32 a				28.7	6.32 a	33.8 b
2012	36.7 c	20.4 b	2.23 ab	15.2 c	33.8 a	5.34 b				26.9	6.34 a	29.3 c
Significance	***	***	*	***	***	***				*ns*	*	***
Rate x year	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*		*		*ns*	*ns*	*ns*

ns, *and *** stand for not significant, significant at p≤0.05 or significant at ≤ 0.001, respectively. In the same column, means followed by the same letter are not statistically different (P ≤0.05, SNK Test). *: interaction between rate and year significant at p <0.05. Values differing by ≥ 2 standard error of means (SEM) are statistically different

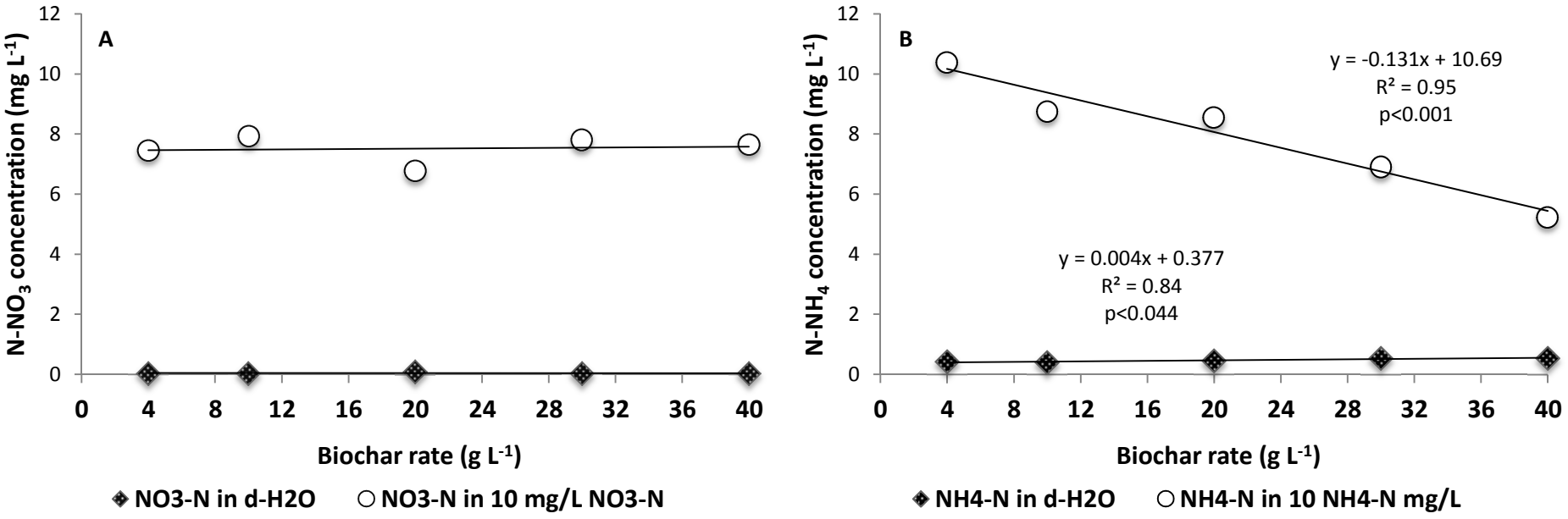


Figure 7.1. Effect of increasing rates of biochar on NO_3^--N (A) and NH_4^+-N (B) release/retention potential

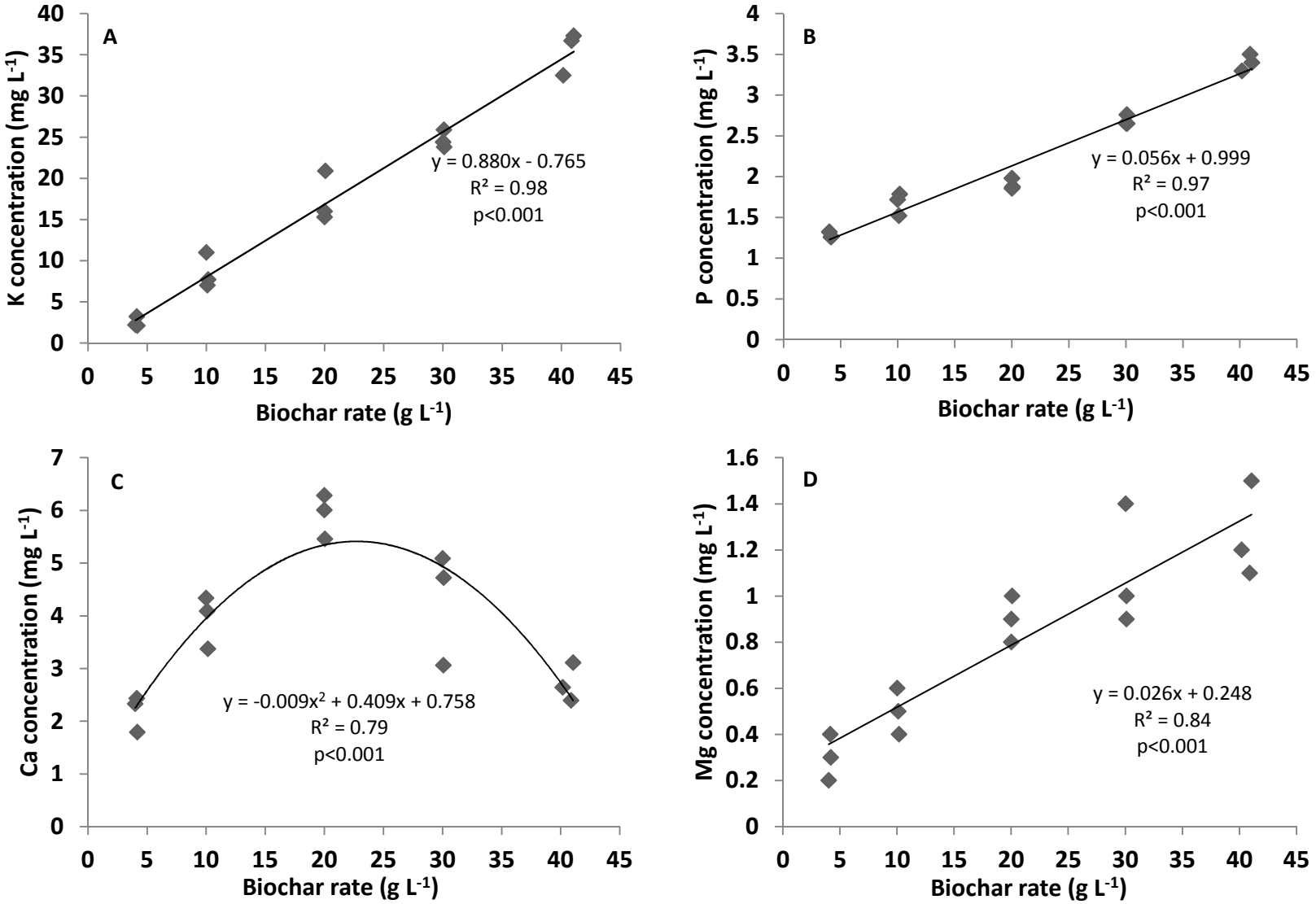


Figure 7.2. Effect of increasing rates of biochar on the release of potassium (A), phosphorus (B), calcium (C) and magnesium (D) in d-H_2O

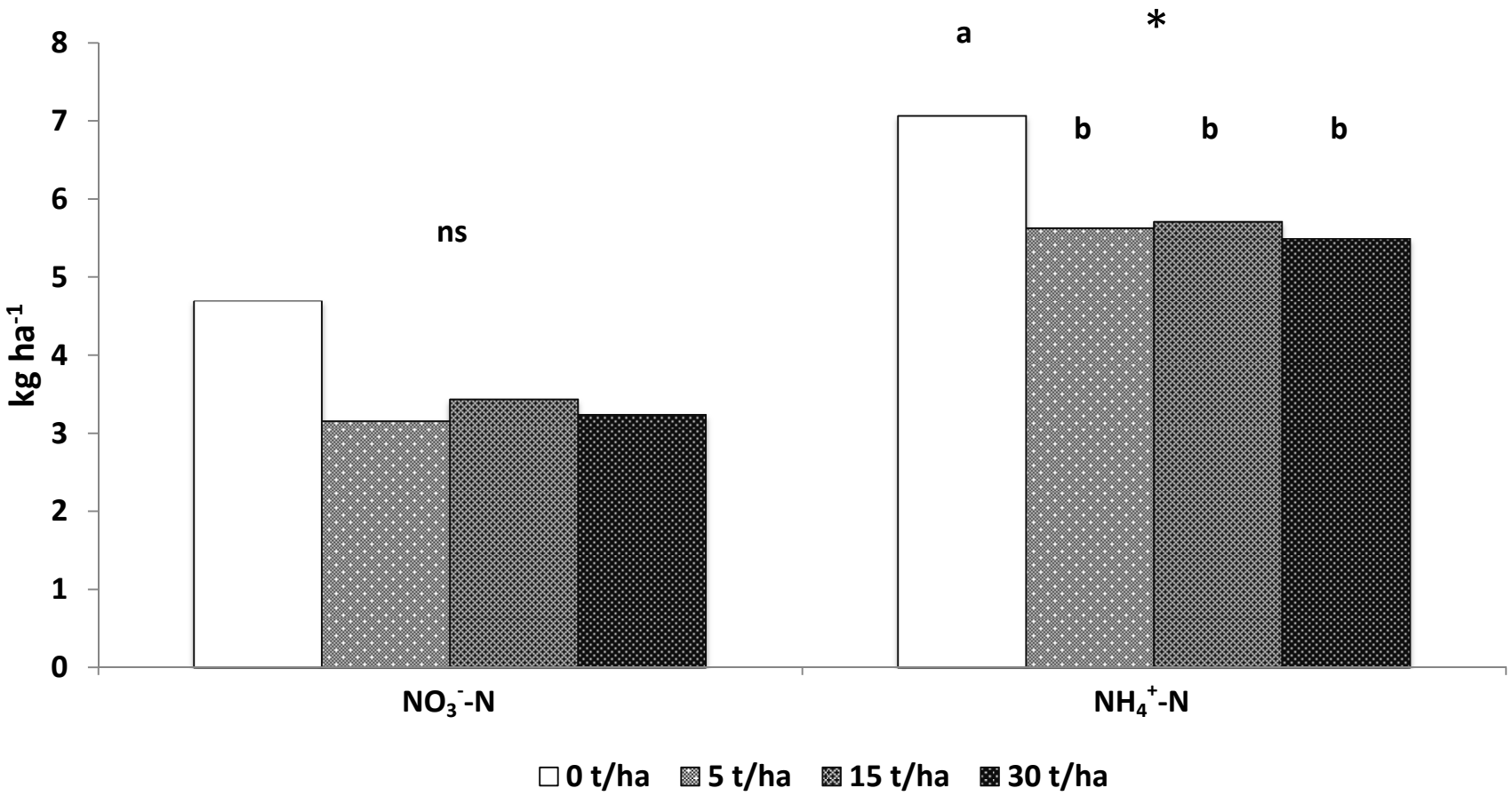


Figure 7.3. Effect of increasing soil-applied biochar rates on cumulative (kg ha^{-1}) nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) leached in 13 months (May 2011 - June, 2012) as recovered by ion-exchange resin lysimeters.

ns and *: effect of rate not significant or significant at $p \leq 0.05$, respectively. Bars with the same letter are not statistically different ($p \leq 0.05$, SNK Test).

CHAPTER 8

Biochar physico-chemical changes as affected by environmental exposure

Abstract

To best use biochar as a sustainable soil management and carbon sequestration technique, we must understand the effect of environmental exposure on its physical and chemical properties because these properties play an important role in its environmental behavior and are likely to vary with time. Here we report physical and chemical changes of biochar fragments recovered from a commercial nectarine orchard after 4 years. We compared fresh biochar with biochar removed from experiments conducted at amendment rates of 5, 15 and 30 t ha^{-1}. We combined a suite of two pycnometry techniques (skeletal (ρ_s) and envelope (ρ_e) density) to estimate the total pore volume of biochar particles. We also examined imbibition, which can provide information about soil hydraulic conductivity. Finally, we investigated the chemical properties, surface, inner layers atomic composition and C1s bonding state of biochar fragments through X-ray photoelectron spectroscopy (XPS).

Ageing increased the overall skeletal and envelope density, while porosity was unaffected. However, water absorption by aged fragments was slower than fresh particles, likely as a consequence of the reduced water accessibility through pores that appeared partially clogged by soil and minerals. Environmental exposure reduced biochar pH, EC and total carbon (C) while XPS analyses showed an increase in total nitrogen (N) and N mineral forms (NO_3-N and NH_4-N) up to 40 nm depth. Ageing resulted in a higher oxygen (O), silicon (Si), N, sodium (Na), aluminum (Al), calcium (Ca), manganese (Mn) and iron (Fe) surface (0-5nm) atomic concentration (at%) and a reduced C and potassium (K) composition, confirming the interactions of biochar with soil inorganic and organic phases. XPS analyses indicated that oxidation occurred on biochar fragments mainly in the exposed top surface, and progressively decreased down to 75nm. Biochar

chemistry changes, as a response of natural oxidation, included the development of O-containing (i.e. carbonyl and carboxylate) functional groups, which we observed mainly in the exposed top surface. However, changes were noticeable also in deeper layers, down to 75 nm while no significant changes were measured in the deepest layer (105-110 nm).

Keywords: Skeletal density, Envelope density, Porosity, Imbibition, XPS, Ageing

8.1 INTRODUCTION

Biochar, the solid residue of biomass pyrolysis, is a highly porous material mainly composed of amorphous C, graphitic (turbostratic) crystallites of polycondensed aromatic sheets and interspersed voids that define its physical structure (Keiluweit et al., 2010). Biochar is deliberately added to crop lands with the goal of effectively sequestering photosynthetically fixed carbon (C), thus potentially mitigating climate changes (Woolf et al., 2010) and ameliorating soil properties (Spokas et al., 2012). An additional goal is often achieving positive crop responses (Verheijen et al., 2010).

8.1.1 Porosity controls many biochar mechanisms in soil

Interspersed voids are responsible for biochar's porosity and are arranged in a complex structure involving interconnected networks of pores (Nguyen et al., 2010; Rouquerol et al., 1994). Biochar pore size range from sub-nanometers corresponding to slit-shaped spaces between graphite-like layers of flat aromatic C clusters (Sun et al., 2012; Keiluweit et al., 2010), to pores of tens of micrometers, reflecting the partially preserved cellular structures (Bird et al., 2008; Wildman and Derbyshire, 1991). Porosity is a major control of biochar sorptive capacities (Karhu et al., 2011; Knicker et al., 2008) and pore size modulates the interactions of biochar particles with microbes, fungal hyphae and plant roots (Warnock et al., 2010; Downie et al., 2009; Thies and Rilling, 2009; Hockaday et al., 2006; Pietikäinen et al., 2000). However, recent findings suggest that the largest pores (>50 microns) are responsible for the vast majority of total biochar porosity (Brewer et al., 2014). In addition, by interacting with water (Brockhoff et al., 2010), biochar macropores may significantly affect soil hydraulic conductivity (Barnes et al., 2014; Oguntunde et al., 2008) and ecosystem processes mediated by water in soils (e.g. infiltration and drainage rates, soil erosiveness, wetting, water holding capacity, amount of plant available water, nutrient leaching) (Baronti et al., 2014; Bruun et al., 2014; Novak et al., 2012; Major et al., 2009). In this sense, density and porosity are essential biochar physical properties controlling the movements of the fragments through the landscape, thereby its soil residence time

(Masiello et al., 2014). Moreover, these properties control habitat for microbes (Ogawa, 1994) and shelters for mycorrhizal fungi (Warnock et al., 2007).

8.1.2 The role of environmental exposure

Given the importance of biochar physical properties (size and porosity) as a possible explanation for many of the biochar induced-effects in soils, it is necessary to understand how these properties change over time as a consequence of long-term environmental exposure. This knowledge can also contribute to our understanding of how the environment affects the fate, transport, and ecosystem services of naturally-produced charcoal (Brewer et al., 2014). Although combined methods can be used to characterize biochar porosity (i.e. gas sorption such as nitrogen (N) and carbon dioxide (CO_2), mercury porosimetry, stereological measurements, BET), every one of these techniques has its limitations (Sun et al., 2012). All the previous methods can be expensive, time-consuming, potentially dangerous during handling or ineffective in accurately measuring the biochar pore volume because biochar pore sizes can range from subnanometer (micropores) (Sun et al., 2012) to pores of tens micrometers or larger (Bird et al., 2008). Since no single technique can precisely measure these pore size scales, biochar porosity characterization has been elusive (Brewer et al., 2014). Similarly, to date no accurate methods have been proposed to investigate the change in rate of biochar pore accessibility over time, in particular as a consequence of the environmental exposure.

Although biochar is predicted highly resistant to decay in soil due to: i) its intrinsic chemical resistance to biotic degradation derived from its condensed aromatic structure, and ii) the tendency of its oxidized surface to form mineral-organic matter complexes (Glaser et al., 2000), biochar does not remain unaltered once in soil, but it undergoes to a series of physico-chemical changes as a consequence of its interaction with the environment (i.e. temperature range, water availability), human activities (tillage, fertilization) and interaction with the soil matrix (i.e. microbes, minerals, OM, roots) (Joseph et al., 2010). Density and porosity of biochar in soil can be significantly altered because its pore network and surface reactivity permit the physical/chemical trapping/attraction of different compounds (e.g. silt, sand, clay, roots, minerals, organic matter, microbes) (Jaafar et al., 2014;

Warnock et al., 2007), with crucial implications for biochar sorption capacity, soil hydraulic conductivity, water-holding capacity and plant-available water.

Changes of biochar properties such as porosity, elemental composition, surface chemistry, absorption of organic C molecules rich in functional groups, adsorption properties, surface acidity and negative surface charges as a consequence of ageing have been reported by many studies (Lecroy et al., 2013; Lin et al., 2012; Jones et al., 2012; Joseph et al., 2010; Zimmerman, 2010; Cheng and Lehmann, 2009). However, most of these findings come from short-period environmental exposure of biochar where often weathering effects were induced through chemical and/or physical treatments (Yao et al., 2010). Little is known about how biochar changes physically and chemically after environmental exposure.

We know even less about how biochar chemistry shifts with depth in the particle. It is not clear how rapidly environmental oxidation proceeds through biochar particles, with some studies suggesting that oxidation is a surface process, and others reporting oxidation throughout the entire particle (Cheng et al., 2006) .It seems reasonable to assume that initially, chemical changes are limited to the surface of the particle, but no information is yet available about changes of distinct internal layers as affected by long-term natural environmental conditions.

In this study we investigated the physical and chemical changes of biochar fragments recovered from a commercial nectarine orchard after 4 years. Our experiments used biochar from a range of amendment rates rates. Recovered particles were compared with fresh fragments selected within the same batch and assessed to evaluate potential shifts in porosity. We combined a suite of two pycnometry techniques (skeletal (ρ_s) and envelope (ρ_e) density) that can successfully and cost-effectively estimate the total pore volume of biochar particles, following the procedure set up by Brewer et al. (2014). Briefly, ρ_s is defined as the volume of a known mass of a biochar particle measured by the displacement of helium (He) that can enter all the connected pores within a particle, leading to the measurement of the solid framework. Envelope density (ρ_e) is the mass of a biochar sample divided by the volume of its non-wetting exterior envelope. The percent of the biochar particle volume not filled by solid, as

calculated from the difference in densities ($1-(\rho_e/\rho_s)$), offers an accurate estimation of the total biochar porosity (Brewer et al., 2014).

We also considered possible implications of biochar ageing on soil hydrologic properties by an imbibition assay. Finally, we measured chemical properties, surface, inner layers elemental composition and C1s bonding state of aged biochar through X-ray photoelectron spectroscopy (XPS).

8.2 MATERIALS AND METHODS

8.2.1 Environmental conditions of the experimental site

For this experiment (2009-13) we used a commercial nectarine (*Prunus persica* L., Batsch) orchard of the cv. Big Top grafted on the hybrid GF677 (*P. persica* L. x *P. amygdalus* L.) planted in 1997 with a density of 519 trees ha^{-1} (3.5 x 5.5 m) located in the South Eastern Italian Po Valley (Tebano, Ravenna, 44° 29' N, 11° 78'E, 58 m a.s.l.). The soil of the orchard was sandy-loam, classified as Inceptisol (USDA, 2010), characterized by a pH of 8.08, an OM content of 10.6 g kg^{-1} a cation exchange capacity (CEC) of 13.0 meq 100 g^{-1} and a total N, assimilable P, exchangeable K, Na, Ca and Mg of 800, 8, 97, 37, 2347 and 109 mg kg^{-1}, respectively.

The experimental area is characterized by a temperate sub-continental climate with cold winters and warm and humid summers. Throughout the experiment, the average air temperature was 13.6 °C with the lowest temperature of -4.1°C recorded in winter 2011 and the highest value of 40.5°C in summer 2012, while annual precipitation ranged between 650 and 910 mm, mainly concentrated in spring and autumn. From May to August trees were drip irrigated and the alleys maintained with native grass species while tree rows (2 m wide) were herbicided with glufosinate ammonium (DL-phosphinothricin), twice per year. Trees were managed in terms of pruning, thinning, fertilization, irrigation as well as pest and disease control according to the regional guidelines of Integrated Crop Management (ICM, 2009). Fertilization was managed by a yearly supply of 0.25 kg N $tree^{-1}$ (130 kg N ha^{-1}) as urea (46% N) at petal fall.

8.2.2 Experimental design

In November 2009 we distributed biochar at the rates of 5, 15 and 30 t fresh weight (fw) ha^{-1} in a complete randomized block design, with 5 replicates of 5 trees each, arranged in 4 consecutive tree rows and leaving at least 10 unamended meters between consecutive plots. Biochar was distributed on a 35 m^2 area per experimental plot (2 m wide along the herbicided strip) and mixed into the first 20-cm soil depth (A horizon) by a disc harrow. Biochar was produced in a traditional charcoal kiln with a mixed feedstock of chipped hardwood (mainly from peach and grapevine), and was slowly pyrolyzed at approximately 500°C. Table 8.1 summarizes physical-chemical biochar characteristics.

8.2.3 Biochar fragment recovery and sample preparation

In November 2013 (4 years after application), we randomly recovered about 50 biochar fragments of different sizes from each replicate (Pic. 8.1). To accomplish this we removed the first 3-5 cm depth soil layer and carefully collected fragments from the soil by forceps, using great care to avoid manual contact or any physical damage to the particles. We immediately sealed the particles in polyethylene bags and transported them to the laboratory in a portable refrigerator.

Control samples of biochar (never field-applied, termed here “fresh”) were stored in hermetically closed plastic bags and maintained in a dry and dark place. A subset of these fresh biochar fragments were processed in the same manner as the soil-recovered biochar fragments (description below).

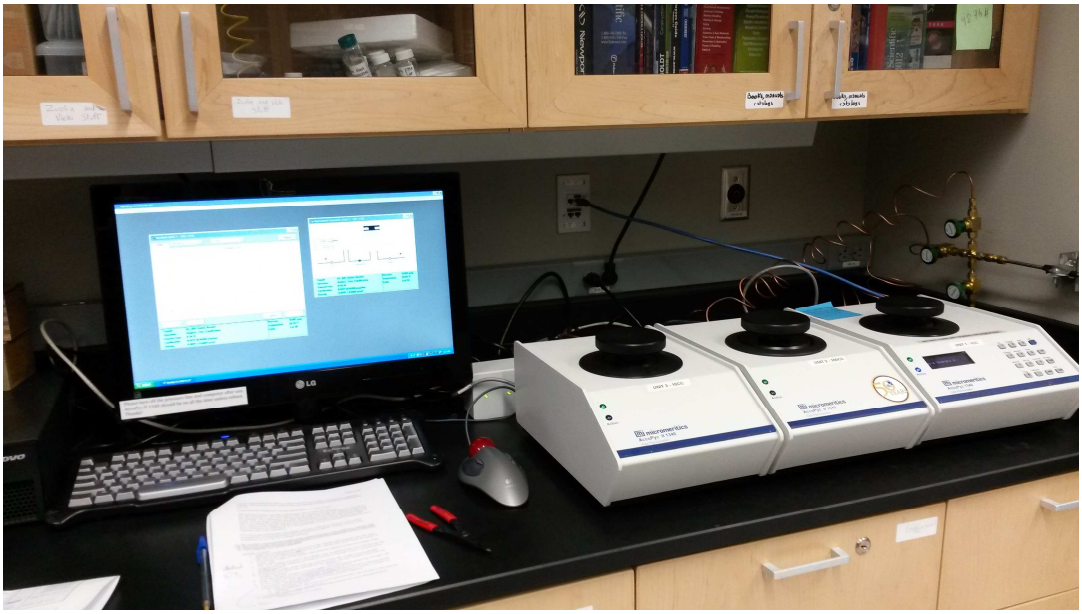
Particles were first dried at 50 °C for few days, sieved (1-mm) to remove excess soil particles and then the surface of individual fragments was gently cleaned with a soft brush and fragments were sparingly rinsed twice with deionized water (d-H_2O) to remove adhering soil from the surface. Fragments were not physically damaged during handling, and were transferred in plastic tubes and oven-dried at 50 °C.

Picture 8.1. Recovering of biochar fragments after 4 years of environmental exposure

8.2.4 Biochar physical changes as affected by the environmental exposure

8.2.4.1 Skeletal density (ρ_s) determination

Skeletal density (ρ_s) represents the volume occupied by a solid sample, including any pores not accessible to He, which is assumed to penetrate all the open pores within the biochar fragment (Brewer et al., 2014). We measured biochar skeletal volume of about 0.1 g (dry weight) per replicate (about 5-6 fragments with each piece larger than 2 mm and smaller than 1 cm^3) using a He pycnometry AccuPyc 1340 (Micromeritics, Norcross, GA) fitted with a 1 cm^3 chamber. The AccuPyc determines the skeletal volume of a sample by measuring the change in pressure due to the volume of He that is displaced by the solid mass within the pressure-equilibrated chamber.



Picture 8.2. The AccuPyc used to measure the particles skeletal density

8.2.4.2 Envelope density (ρ_e) determination

Envelope density (ρ_e) is the sample mass divided by the total sample volume that is measured if an "envelope" were placed around each individual particle. We measured biochar envelope volume of about 0.215 g (dry weight) per replicate (about 8-9 fragments with each piece larger than 2 mm and smaller than 1 cm^3) by a Geopyc 1360 Envelope Density Analyzer (Micromeritics, Norcross, GA) (Pic. 8.3). Briefly, fragments were placed in a bed of DryFlo® granular medium (density of ~0.4 g cm^{-3}), gently consolidated around the biochar particles to a force of 22 N using a piston sliding on a 12.7 mm diameter chamber. Sample volume was determined by subtracting the volume of the consolidated pure DryFlo® from the volume of the same consolidated DryFlo® after the sample addition. Consolidation was achieved by continuous rotation and vibration of the cylindrical chamber as the piston was gradually pushed into the chamber until the stated 22 N force was reached.

Picture 8.3. The Geopyc allowed the measurement of the particles envelope density. On the right, detail of the glass chamber where a sliding piston consolidated the pure DryFlo® around the biochar fragments

8.2.4.3 Porosity determination

Porosity is defined as the percent of the biochar volume not filled by solid including pores smaller than the DryFlo® granules and pores that are inaccessible from the exterior of the biochar surface. Porosity is a function of skeletal and envelope density and was calculated as follow:

$$\varphi = \frac{v_e - v_s}{v_e} = 1 - \frac{m/\rho_s}{m/\rho_e} = 1 - \frac{\rho_e}{\rho_s}$$

where:

φ = porosity

v_e and v_s = envelope volume and skeletal volume

m = mass

ρ_e and ρ_s = envelope density and skeletal density

8.2.5 Imbibition assay

For this assay, we compared fresh vs. aged biochar fragments from the 30 t ha^{-1} plots. Samples were treated as previously mentioned and three pairs of fragments were selected, with pairs having similar weight (± 0.04 mg) and shape. Samples were gently rinsed with d-H_2O and then oven dried at 50 °C. The former step was repeated 3 times to reduce hydrophobicity. Fragments were individually transferred into glass tubes containing 6 cm (75 mL) of d-H_2O. Each couple of fragments was

simultaneously and carefully placed on the water surface and let to float. Tubes were not hermetically sealed, never shaken or disturbed throughout the test and maintained at room temperature (21 °C). Fragments were allowed to naturally absorb water and we recorded the sinking dynamics of each fragment at 12 hr intervals until particles reached the bottom of the tubes. Thereafter, fragments were carefully removed and the amount of absorbed water was measured by massing before and after drying at 105 °C.

8.2.6 Biochar chemistry changes following environmental exposure

8.2.6.1 pH and electrical conductivity

We determined biochar pH and electrical conductivity (EC) on entire fragments. Samples were oven dried (105°C), then 0.5 g were added to 10 mL of d-H_2O in plastic tubes and shaken 1 h at 120 rpm. pH and EC were measured on the filtrated surnatant under continuous stirring by a pH-meter (BasiC 20, Crison, Barcelona, Spain) and a conductimeter (CDM210 Conductivity Meter, Radiometer Analytical, Copenhagen, DK).

8.2.6.2 Total C, N, H content

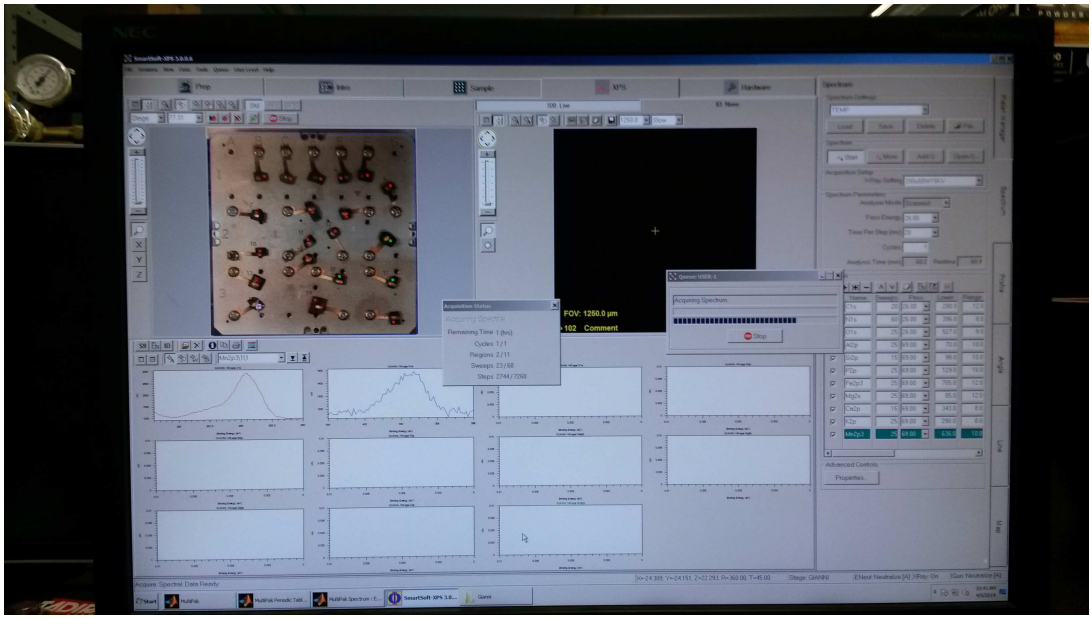
We manually milled and homogenized fifteen biochar fragments per replicate using a mortar, then sampled 3 mg for total nitrogen (N) and hydrogen (H) and 0.1 mg for C determination via catalytic combustion analysis (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA) at 2.33 mV voltage. Retention time was 1.21, 1.78 and 5.47 min for N, C and H respectively. Data were compared with external calibration curves at 9 points ($r^2 > 0.9999$) obtained by a high-purity acetanilide standard (Costech Analytical Technologies Inc., Valencia, CA).

8.2.6.3 KCl extractable NO_3^--N and NH_4^+-N

We extracted intact oven-dried biochar fragments using a 2 M KCl solution at a ratio of 1:20 (w w^{-1}). Samples were shaken 90 min at 100 rpm by an orbital shaker and the filtered (Whatman, 42) supernatant was analyzed for nitrate-N (NO_3^--N) and ammonium -N (NH_4^+-N) concentration by an autoanalyzer (Auto Analyzer AA-3; Bran+Luebbe, Norderstadt, Germany).

8.2.6.4 Surface atomic composition

We analyzed three fragments per replicate by X-ray photoelectron spectroscopy (XPS) to determine the relative C, oxygen (O), silicon (Si), N, sodium (Na), aluminum (Al), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn) and iron (Fe) atomic concentration (at%) in the top 5 nm biochar surface (Fig. 8.1), using a PHI Quantera XPS with a focused monochromatic Al Kα X-ray source for excitation operated at 1486.6 eV and 49.2 W. We performed high resolution and low intensity scans to focus on the C bonding environments with 40 scans. XPS spectra were analyzed using a nonlinear least-squares curve-fitting program with a Gaussian−Lorentzian mixed function to optimize the spectra. We analyzed spectra using MultiPak data analysis software (MultiPak V7.0.1, 04 Mar 16, Ulvac-Phi, Inc., 1994-2004).



Picture 8.4. The XPS software scanning the surface of the biochar fragments

8.2.6.5 Biochar inner layer atomic composition

We compared, with four replicates, fragments of fresh and aged biochar (recovered from plots treated with 30 t ha^{-1}) by XPS to determine the atomic concentration (at%) for relative C, O, Si, N and Al at four depths (S1=0-5nm, L2=5-10nm, L3=15-20nm and L4=30-35nm; Fig. 8.1).

An additional set of three fragments of fresh and aged biochar (recovered from plots treated with 30 t ha^{-1}) were used to determine the atomic composition (at%) for relative C, O, Si, N and Al at three supplementary depths in addition to the top surface layer (S1=0-5nm, L5=35-40nm, L6=70-75nm and L7=105-110nm; Fig. 8.1) using the same methodology as described above. For both set of samples we analyzed spectra and deconvoluted the C1s region bonding state into their component functional groups using MultiPak data analysis software (MultiPak V7.0.1, 04 Mar 16, Ulvac-Phi, Inc., 1994-2004). The -C-C/-C-H/-C=C bonds exhibit the same binding energy (284.74 eV), thus were considered together, while -C-O, -C=O and -COOH were targeted at 285.95, 287.18 and 288.56 eV, respectively.

8.3 STATISTICAL ANALYSES

Data were evaluated by analysis of variance (ANOVA) according to a complete randomized design with 5 replicates. Data from XPS analyses were evaluated by ANOVA according to a complete randomized factorial design with 2 factors: biochar rate (4 levels) and layer (4 levels). When ANOVA showed a statistical effect (at $P \leq 0.05$), means were separated by Student-Newman-Keuls Test (SNK); when interaction between age and layer was significant, 2 times standard error of means (SEM) was used as the minimum difference between two statistically different means (Saville and Rowarth, 2008). Data of the imbibition assay were submitted to repeated measures analysis of variance using PROC MIXED (Littel et al., 1998) in SAS software (SAS Institute Inc., Cary, NC, USA), with the fragment weight as covariant and a compound symmetry covariance structure.

8.4 RESULTS

8.4.1 Biochar physical changes as affected by environmental exposure

8.4.1.1 Skeletal, Envelope density and Porosity

Biochar skeletal density increased only when applied at rates higher than 5 t ha^{-1}, reaching values higher than 2 g cm^{-3} (Fig. 8.2). The lowest application rate (5 t ha^{-1}) showed intermediate values between the higher aged rates (15 and 30 t ha^{-1}) and fresh biochar (Fig. 8.2). The skeletal density of fresh biochar was 1.86 ± 0.04 g cm^{-3} (avg. ± SE).

Although the environmental exposure overall increased the envelope density of biochar fragments, it only resulted in significantly higher values when biochar was applied at the rate of 15 t ha^{-1} compared to fresh fragments (Fig. 8.2). Fresh biochar envelope density values were 0.246 ± 0.006 g cm^{-3} (avg. ± SE).

Total porosity was unaffected by environmental exposure and values were 86.8 % ±0.01 (avg. ± SE) (Fig. 8.2).

8.4.1.2 Imbibition assay

The sinking dynamics of fresh and aged biochar fragments were significantly different (Fig. 8.3). Fresh biochar samples started to sink after 156 hrs, then steadily continued, reaching the bottom of the tubes between 162 and 168 hrs. (Fig. 8.3). Aged fragments floated significantly longer (Fig. 8.3), suggesting pore blockages had trapped air in macropores. For the aged samples sinking started between 168 and 180 hrs, continuing slowly up to 268 hrs, and then sinking was faster and fragments reached the bottom of the tube after 276 hrs. (Fig. 8.3). The ratio of water:biochar (w w^{-1}) of the sunken fragments was unaffected by ageing and values were 4.98 (±0.30 n=3) and 5.16 (±0.35 n=3) for fresh and aged fragments, respectively.

8.4.2 Biochar chemistry changes as affected by the environmental exposure

8.4.2.1 pH, EC, total elemental C, N, H, extractable NO_3^--N and NH_4^+-N concentration

Ageing statistically decreased pH and increased EC, and showed no differences between biochar application rates (Tab. 8.2). Total C concentration was

significantly reduced in aged biochar fragments on average by 14.5 % (±0.0.18 n=5), with a comparable extent among rates, while no differences were observed for H concentration (Tab. 8.2). Conversely, fragments exposed to the environment showed a significantly higher total N concentration compared to fresh particles (Tab. 8.2), although the intermediate rate (15 t ha^{-1}) showed a lower value in comparison with other rates (Tab. 8.2). Independently of the rate, N mineral forms (NO_3^--N and NH_4^+-N) extracted from aged biochar were significantly increased (Tab. 8.2) with respect to fresh biochar.

8.4.2.2 Biochar surface atomic composition

Ageing significantly affected the surface relative atomic composition (Fig. 8.4). Biochar surface C and K atomic composition was reduced compared to fresh biochar by 30 and 87% on average, respectively, occurring at a similar extent among all biochar application rates (Fig. 8.4). Similarly, surface atomic composition of Al, Si, Ca, Mn and Fe were higher in aged fragments without any effect induced by the application rate (Fig. 8.5). The lowest values of atomic O composition were recorded in fresh fragments, while aged particles from the highest biochar application rate showed intermediate values (Fig. 8.4). Except when biochar was applied at the lowest rate (5 t ha^{-1}), values of surface N atomic composition were significantly higher in aged fragments, whereas surface Mg and P concentrations were unaltered in all treatments (Fig. 8.4).

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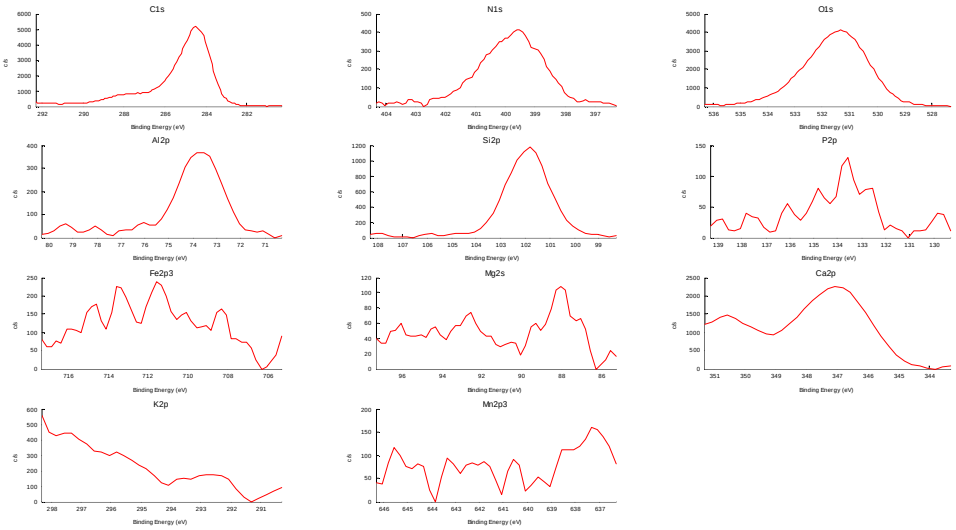
2014 Apr 9 Al mono 49.2 W 200.0 µ 45.0° 26.00 eV 7.0219e+002 max 3.20 s
C1s/10: 30T1/1 (SG5 Shft)

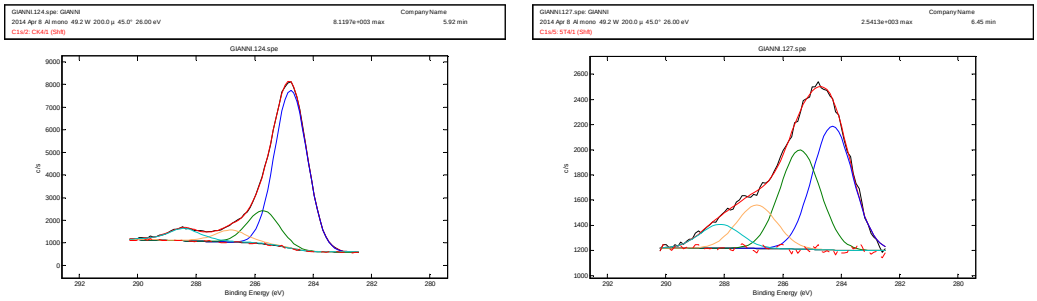


Picture 8.5. Chromatograms obtained by scanning the biochar surface through XPS

8.4.2.3 Biochar atomic composition up to 35 nm depth

Without interaction between factors (depth and ageing) values of biochar C, Al, O, and Si atomic composition were statistically comparable between surface and inner layers (Tab. 8.3). However, values were significantly altered by ageing. Atomic C composition was reduced in aged fragments, in contrast with Al, O and Si which increased, consistent with the presence of soil minerals (Tab. 8.3). Ageing and depth interacted for N atomic composition, which was higher in aged fragments only up to the second layer (10 nm depth), while it was similar to values of fresh biochar samples in the two deeper layers (<35 nm) (Tab. 8.3).

No interaction was observed between depth and ageing in Cs1 functional groups (data not shown). However, relative atomic percentage of C functional groups -C-O, -C=O and -COOH increased in weathered samples, while -C-C/-C-H/-C=C bonds decreased (data not shown). In addition, depth did not affect the relative atomic percentage of -C-C/-C-H/-C=C while C=O and -COOH functional groups were significantly higher only in the top surface as compared with inner layers. An opposite trend was recorded for the -C-O group, which increased with depth (data not shown).



Picture 8.6. C1s region bonding state deconvoluted chromatograms. On the left, spectra of fresh biochar as compared with an aged (30 t ha^{-1}) biochar fragment (right).

8.4.2.4 Biochar atomic composition up to 110 nm depth

Ageing and depth interacted for biochar atomic C and N composition (Tab. 8.4). All four analyzed layers of aged biochar showed a significantly reduced C atomic composition independent of depth (Tab. 8.4), while only in the top surface (0-5nm), atomic C composition of fresh biochar was lower than inner layers. C of aged fragments was reduced in the first two layers (up to 40 nm) compared to deeper layers (Tab. 8.4).

Ageing and depth significantly interacted with atomic N composition up to 75 nm depth (L6), while no interaction was recorded between the two deepest layers (Tab. 8.4). Atomic N composition was significantly higher in the first two aged layers compared whit the fresh biochar and it was significantly reduced in the aged fragment as the depth increased among the top 3 layers (Tab. 8.4). Ageing and depth did not interact in atomic O, Al and Si composition (Tab. 8.4). Independently of the layer, values resulted always significantly increased in aged biochar by 3, 5 and 18 fold, respectively (Tab. 8.4). Depth only affected atomic O composition, which was reduced as the depth increased (Tab. 8.4).

The two factors (ageing and depth) always interacted with C functional groups (Tab. 8.5).

The relative atomic percentage of the different C functional groups was always increased by ageing in the first 3 analyzed layers (0-75 nm) (Tab. 8.5), except for the -C-C/-C-H/-C=C bonds, where only in the top surface an opposite trend was

recorded (Tab. 8.5). No differences were measured in the deepest layer (105-100 nm) between fresh and aged biochars (Tab. 8.5).

8.5 DISCUSSION

8.5.1 Ageing increased skeletal and envelope densities

Once incorporated within the soil, biochar physical-chemical properties are expected to change with time and a reduced porosity of biochar fragments has been indicated among possible outcome of ageing in soil (Barnes et al., 2014). This is because biochar in soil undergoes to a range of biogeochemical interactions, including a series of physical processes (e.g. tillage, freeze-thaw, rain and wind, etc.) that may alter particle size, pore connectivity, chemical composition and sorption capacity (Spokas et al., 2014; Hammes and Schmidt, 2009). As mentioned, the skeletal density is the volume measured by the displacement of helium (He) that can penetrate virtually all the connected pores within a known mass of a biochar particle while the envelope density represents the volume of a solid material displaced by biochar.

In our experiment, 4 years of environmental ageing increased both biochar skeletal and envelope densities (although not always statistically significantly) without a clear trend induced by the rate of application. Compared to fresh particles, the most significant change induced by ageing was observed for skeletal density, which increased on average by 120 mg cm^{-3}, while the envelope density increased by 20 mg cm^{-3}. This response may be due to either structural cracking that occurred in biochar particles over time as a consequence of mechanical stresses that led to alteration of the original pore connectivity, and/or to the interactions of biochar with the soil mineral and organic phases. Surface cracks and externally connected pore opening represent points of entry for solid particles (soil minerals, organic compounds, biochar ash or residues) dispersed in the soil solution, allowing access from the biochar surface into its core. Once inside, these particles may flow in the internal pore network flushed by water movements. Soil particles in pores <20 μm of a biochar recovered from a 2-month incubation (Jaafar et al., 2014) and clay and silt were distinctly identifiable in the macropores of an aged sectioned greenwaste biochar through SEM images (Joseph et al., 2010). Our microscopic images revealed that minerals partially filled biochar fractures starting from its outer faces.

Biochar fragments appear wrapped by soil particles that either adhered to the rough surface or were chemically retained on our recovered biochar. Images show that despite our effort to remove and separate uncharred particles from biochar fragments, a small amount of soil was observed on surface and within pores that potentially affected also biochar chemical analyses.

Although biochar pore sizes occur over many orders of magnitude (Brewer et al., 2014), plant-derived biochars typically have a high concentration of macropores with a diameter >1 µm (Downie et al., 2009). This size fraction includes pores larger than a water molecule (0.28 nm). Capillary forces may also drive the soil solution into biochar pores, carrying small mineral and organic particles in suspension (including small C-sheets resulting from biochar physical cracks) into biochar microvoids that may accumulate (and/or clog) in the pore channels (Joseph et al., 2010). Charred and non-charred compounds can then remain physically blocked or chemically attracted in the internal biochar voids, altering its original framework, hence pore connectivity. We observed minerals and organic residues in internal pore channels of aged sectioned fragments. Some particles were physically trapped in pores, totally or partially clogging their access. Brodowski et al. (2006) and Liang et al. (2006) suggested a close interaction between biochar particles and clay mineral surfaces which may lead to the occlusion of biochar pores, limiting the accessibility to inner voids (Warnock et al., 2007). If any particle would clog the only access of a "dead-end" pore, this would cause a large decrease in the skeletal density, since He cannot invade the isolated volume. In the environment these newly-inaccessible volumes may be occupied by a combination of trapped water and/or air, leading to porosities that vary with water exposure history. Biochar densities and porosities measured here after drying at 105°C should therefore be considered upper limit values.

The extent at which particles enter the biochar pores depends to a first order on pore size, and then on the macropore diameter, connectivity, length and tortuosity. As a consequence of environmental exposure, oxidized biochar particles may be bound with clay and silt-sized minerals and this association can increase the ability of the soil-biochar complex to sorb organic compounds in soil (Browdowski et al. 2006). Direct sorption of organic matter onto biochar surfaces in soil was also

indicated by Uchimiya et al. (2010). The attachment of organic compounds on the biochar surface was considered to be among the factors responsible for the decline in the sorption capacity of aged biochar, since micropores appeared clogged (Pignatello et al., 2006).

Biochar physical properties can be also altered by its interaction with microorganisms in soils (Hockaday et al., 2006). In a 56-day incubation experiment, biochar retrieved from soil exhibited larger pores distinctly colonized by fungal hyphae which were observed across the entire biochar particles (externally) and within pore spaces (Jaafar et al., 2014). Microbes may clog internal pores over time and appear in pycnometric measurements as solids inaccessible to He displacement, thus contributing to a decrease biochar density.

8.5.2 Agronomical and ecological implications

Even though both biochar skeletal and envelope densities increased as a consequence of environmental exposure, porosity, defined as the ratio of these, was unaffected, suggesting that both fresh and aged particles may show similar porosity-driven hydrologic behavior. Nevertheless, the imbibition assay showed that water absorption kinetics of aged biochar were significantly slower than those of fresh fragments. The slower water infiltration experienced by aged particles is likely due to the reduced water accessibility of pores and fractures, suggesting that the effect of biochar on soil hydrology may change with time. However, this response raises a number of questions. For example, it seems reasonable to assume that different soil textures and mineralogies will interact differently with various biochars and thus biochar and ecosystem-specific patterns of sealing exterior pores may be expected.

Recently, two hydrologic pathways were proposed to be potential drivers for hydraulic conductivity in the soil-biochar mixture (Barnes et al., 2014): the first includes the interstitial space between biochar and soil (interparticle spaces) and the second is represented by the voids within the biochar grains themselves (intraparticle spaces). In our experiment, the total amount of water absorbed by each particle (intraparticle) once sunk was unaffected by environmental exposure, suggesting that after 4 years in soil the potential of biochar to retain water remain

unchanged, while the speed at which water penetrated within particles was much slower. This shift has several implications for soil processes, both in processes mediated by water in soils as well as in the erosive fate of aged particles in the environment.

Porosity is a major control on biochar sorptive capacities and that the ability to adsorb water and nutrients is thought to be one of its most environmentally valuable properties (Brockhoff et al., 2010; Downie et al., 2009). Our water imbibition kinetics show a lag in aged biochar, suggesting that older biochar may take longer to sorb water and may retain water longer, potentially at higher water potential values. A shifting in soil water infiltration and drainage rates may be then hypothesized after few years upon biochar addition. For this reason, soil leaching may result different in aged biochar-mixed soils compared to the immediate response of biochar addition under continuous irrigation or heavy rain events.

Furthermore, the influence of biochar on water holding capacity and amount of plant available water may change as biochar ages, in particular in easily drained soils (e.g. coarse sand) and especially if a dramatic reduction occurs in the number the pores between 0.1 and 50 µm. This pore size range in biochar is fundamental to increased plant available water since larger pores weakly retain water under gravity (Jury et al., 1991) and nanometer-scale pores do not provide water in a plant-accessible form (Masiello et al., 2015).

Water infiltration shifts the envelope density of biochar as water fills internal pores previously occupied by air. Once water-filled, the envelope density of biochar particles is higher than that of water, leading to sinking. The time dynamics of this sinking process seem to be altered by field aging, with mineral blockages of pore throats slowing the rate of water infiltration. This lag in particle infill time has implications for the erosion rate of biochar particles. We hypothesize a threshold effect, with aged biochar particles taking longer to dry out, leading to slower erosion rates. However, once biochar particles have become completely dried, they may take longer to refill, leading to more rapid erosion. This process likely has a pedogenic endpoint that occurs when biochar particles become deeply enough embedded within the soil matrix that their dry envelope density exceeds 1 g cm^{-3}.

Our experiments suggest that this endpoint was not reached after 4 years for the particular biochars and soils used in this experiment.

The porous structure of biochar provides suitable habitat for a range of microbial communities (Downie et al. 2009; Thies and Rilling, 2009; Warnock et al., 2007; Hockaday et al., 2006), and fungi can grow from within the pores out into the soil Ogawa (1994). As biochar interacts and ages in soil, microbes can enter and inhabit biochar pores. Attachment of soil particles to biochar surfaces may also alter habitat suitability and microbial activity (Thies and Rilling 2009; Lehmann et al. 2011).

Pore connectivity has been suggested to modulate the availability of biochar-associated labile organic compounds to microbial enzymes (Barnes et al., 2014). Easier access to these sites in recently added biochar could partially explain the initial high mineralization rates observed after biochar addition (Cross and Sohi, 2011).

Minerals covering the external surface of biochar fragments interfere with its reactive surface, limiting the sorption capacity (i.e. for organic compounds; Joseph et al., 2010) but at the same time the greater reactivity of the surface due to oxidation may promote physical protection of biochars and, thus, its long-term stability (Brodowski et al., 2006). Our results document the timescale of mineral adsorption to biochar surfaces in this sandy-loam Inceptisol.

8.5.3 Biochar chemical changes as affected by ageing

Although chemically-induced biochar degradation starts before its incorporation in soil as a result of the oxidation of exposed C rings with a high density of π-electrons (Contescu et al., 1998) and free radicals (Montes-Morán et al., 2004), only once in soil does biochar experience significant weathering. Persistent residence in soil alters the chemistry of biochar and thus changes in soil properties induced by biochar application are likely to evolve with time. The main chemical changes found to occur during biochar aging are shifts in elemental composition, surface chemistry, and adsorption properties (Cheng and Lehmann, 2009). Different processes (dissolution, hydrolysis, carbonation, decarbonation, hydration, redox reactions) and several mechanisms (H-bonding, cation-bridging, covalent

bonding and hydrophobic types of interactions) are involved in biochar weathering processes as a consequence of its interactions with OM, water, adsorption of dissolved organic (e.g. root exudates) and inorganic compounds and oxidation (Joseph et al., 2010).

Except for C and K, the relative atomic surface (0-5 nm) composition of aged biochar increased for most of the investigated elements, while only P and Mg were unaltered. This response can be ascribed either to physical or chemical mechanisms. In fact, the surface of the weathered biochar particles was finely coated with soil and organic residues which appeared adhering and/or trapped in pores and fractures, partially explaining the higher concentration for most of the elements found on the biochar surface. Chemical mechanisms involve the high reactive charge density of the biochar surface (Van Zwieten, et al., 2010) which has adsorption sites where cations, clay and organic matter may be bound by ion and covalent bindings, confirming the interaction of biochar with minerals and organic compounds in soils. The potential of biochar to retain minerals directly on its surface (Glaser et al., 2002) increases the ability of biochar to retain nutrients in soils. Various combinations of Al, Si, C, Fe, and Ti, and trace amounts of Ca, Mg, Mn, K, Na, P, and S, were found at the external surfaces of aged greenwaste biochar particles (Joseph et al., 2010). However, the lack of change in the P and Mg atomic surface composition found in this study indicates that this process is biochar-type and soil dependent.

The decline of total C concentration in aged biochar is due in part to the mineralization of the labile C-fraction associated with biochar (Norwood et al., 2013). In fact, the biochar C-phase exhibits a high concentration of both aromatic and aliphatic regions (Joseph et al. 2010). The first is relatively stable, whereas the aliphatic C regions (volatile organic compounds originated during pyrolysis and condensed during cooling; Rajkovich et al., 2012) are more reactive (Joseph et al. 2010). This fraction leads to an initial evolution of biochar-derived CO_2 in soils after its application (few months), partly attributed to biochar surface oxidation (Bruun et al., 2008; Steiner et al., 2008). However, the increase in aromaticity of the dissolved organic C (DOC) measured in the leachate upon biochar addition suggests that a portion of the labile biochar-derived C can be lost through

percolation (Barnes et al., 2014). Compared to deeper layers (up to 100 nm), we saw a relative C loss in fresh biochar only in the top 5 nm layer, as a response of the natural oxidation occurred on the surface. However, the relative atomic C composition of aged biochar was reduced up to the layer between 40 and 70 nm, indicating that exposure in croplands strongly alters biochar C surface composition. In our experiment after 4 years biochar lost about 15% of its initial total C content. However, although higher and faster C-losses have been documented (Rogovska et al., 2011) the amount of labile C lost compared to stable C stored in soils with biochar is still considered comparatively negligible and should not affect the C sequestration potential of biochar on a long-term basis (Joseph et al., 2010).

We found total N on recovered biochar particles to be 4-fold higher than fresh fragments, independent of the application rate. The most significant contribution to the total N increase was due to the organic N forms, which were 56% of the total N, on average. Similarly, Joseph et al. (2010) reported a general increase in the N content of two different biochars. This was shown to be mainly associated with proteins, amino acids, NH_4^+ and N–C compounds.

Mineral N released by biochar was mainly in the form of NH_4^+. However, the extractable NO_3^--N and NH_4^+-N concentration was 14 and 2 times higher in aged than in fresh biochar, respectively, indicating the potential of biochar to retain both mineral N forms. This confirms the potential effect of biochar on N retention and in reducing the emissions of N-containing GHGs in soils (Spokas et al., 2012). N atomic composition was found to significantly decrease as depth increased up to 75 nm, suggesting that mechanisms for N retention are not limited to the top surface.

We observed that K atomic concentration was significantly reduced in aged biochar surfaces by 87% on average compared to the initial composition. In fact, dissolution of soluble salts and organic compounds (i.e. biopolymers and low molecular weight compounds) associated with charred particles is among the first reactions upon biochar addition to soil (Joseph et al., 2010; Shinogi et al. 2003). This is also confirmed by the reduced CE that we observed in aged particles. The dissolution process may induce a rapid increase in the availability of water soluble cations in the soil layer, where biochar is incorporated, thus when high rates are applied, biochar may represent a consistent source of K, enough to fulfill plant

requirement for the first 2-3 seasons after its incorporation. However, results from a column experiment showed that weathering reduced not only the content of K but also S, Ca, and P (Yao et al., 2010), suggesting that mineral release from charred materials is controlled by biochar characteristics and the environment.

As expected, extensive oxidation occurred on the aged biochar surface. Although not statistically significant, oxidation was more evident mostly on the exposed top surface, and was progressively less pronounced as depth increased down to 35 nm. On the other hand, independently of the environmental exposure, atomic O composition in aged biochar significantly decreased down to 75 nm depth.

Results showed that the O:C ratio of our biochar surface shifted from <0.074 to >0.58 after 4 years in field conditions as a consequence of the depletion of C and increase of O content. This may have consequences for biochar stability in soil, since the increase of the O:C ratio has been cited as a fundamental attribute in controlling the resistance to microbial mineralization (Spokas et al., 2010; Harvey et al., 2012), although it may also simply reflect the increased O present in soil minerals which have attached to the biochar surface.

8.5.4 Ageing promotes surface C functional groups

Biochar chemistry changes induced by environmental exposure include the development of carbonyl, carboxylate, ether, and hydroxyl C functional groups, which are also responsible for the increase in CEC as biochar ages (Cheng et al., 2008; Mao et al., 2012). In our study, an overall development of C functional groups (-C=O, -C-O, -COOH) on the aged biochar surface was observed as a consequence of the natural oxidation which involves the increase in O and H composition (Qian and Chen, 2014; LeCroy et al., 2013; Lin et al., 2012; Jones et al., 2012; Yao et al., 2010; Cheng et al., 2008). This oxidation is attributed to both biotic and abiotic processes, although some data suggest that biotic processes dominate (Zimmerman, 2010; Cheng et al., 2006). The increased oxidation of C in the uppermost surface layers of the aged biochar confirms that oxidation and/or adsorption of soil OM occurred (Joseph et al., 2010). Nevertheless, different functional groups can be formed on aged biochar through oxidation such as, lactonic, o-quinone-like structures and ether-type oxygen (Boehm, 2001). In our case, the -C-C/-C-H/C=C bonding state was always the major component of both

fresh and aged biochar, although after 4 years the relative composition of these C bonds significantly decreased only in the top surface.

The most significant changes in the C1s bonding state were evident on the top surface (0-5 nm), where the relative concentration of -C=O, -C-O and, although to a lesser extent of -COOH, was significantly higher in aged biochar. It is possible that carboxyl functional groups were less developed relative to other oxidized carbon forms because carboxyl groups may be partially decarboxylated through hydrolysis reactions occurring in solution (Yan et al., 1996).

The development of O-containing C functional groups increases the reactivity of the biochar surface, leading to an enhancement of chemical sites able to retain nutrients and other organic compounds on this surface. This process is also responsible for the evolution of negative charges, raising the biochar CEC over time (Zimmerman, 2010).

Oxidized biochar particles may then be bound to soil minerals. Mineral attachment has been indicated as one of the possible mechanisms for the slowing of biochar decomposition and oxidation (Nguyen et al., 2008; Browdowski et al., 2006), acting as a control on the stabilization process of charred particles.

Weathering processes, and in particular the development of carboxylic acids functional groups, lead to a decrease in the concentration of basic sites on the biochar surface (Yao et al., 2010; Cheng and Lehmann 2009; Cheng et al., 2008) which can explain the observed significant reduction of pH (~2 units) in aged biochar. This suggests that the liming potential of biochar may be limited over time. Hence, biochar-induced benefits in nutrient availability in acid soils may be more pronounced in the first seasons following application. For the same reason, the undesirable further pH increase in alkaline soils due to biochar application may be transient.

8.6 CONCLUSIONS

Our results showed that 4 years of exposure in field conditions increased both biochar skeletal and envelope density and, although total porosity was unaffected, water infiltration within aged particles was significantly slower, likely due to the reduced accessibility of water in pores and fractures. This has implications for soil hydraulic conductivity, biochar movement in the environment and in other processes mediated by water in soil, including soil water-holding capacity and plant-available water. Biochar porosity itself does not seem enough to predict the long-term effect of biochar on the hydraulic response of the soil-biochar mixture. Measures of pore accessibility may also be needed. Ageing decreased biochar C and K content but increased the overall relative mineral composition for Si, Al, Ca, Mn and Fe in the topmost layers of the biochar surface (0-5 nm), confirming the interactions of biochar with soil inorganic and organic phases. Similarly, both organic and mineral N content increased in aged biochar up to 40 nm depth. Biochar chemistry changes, as a response of natural oxidation, included the development of O-containing (i.e. carbonyl and carboxyl) functional groups, which were observed mainly in the exposed top surface. However, changes were noticeable also in deeper layers, down to 75 nm while no significant changes were measured in the deepest layer (105-110 nm).

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Table 8.1. Physico-chemical properties of the biochar used in the experiment

Parameter	Unit	Value
Physical properties		
Moisture	g 100 g^{-1}	13.8
Bulk density	g cm^{-3}	0.43±0.04
Hydrophobicity		Slightly hydrophobic
Total porosity	mm^3 g^{-1}	2722
Transmission pores	mm^3 g^{-1}	318
Storage pores	mm^3 g^{-1}	1997
Residuals pores	mm^3 g^{-1}	406
Max water absorption	g g^{-1} of dm	4.53
Skeletal density (SD)	g cm^{-3}	1.86±0.04
Envelope density (ED)	g cm^{-3}	0.2459±0.0056
Porosity (ED/SD)	%	0.863±0.006
Surface area[1] (BET Brunauer–Emmett–Teller method)	m^2 g^{-1}	410±6
Particle size distribution[1]	mm g^{-1}	
50-20	%	4.45
20-10	%	12.1
10-8	%	13.1
8-4	%	10.36
4-2	%	19.85
2-1	%	24.2
<1	%	15.94
Chemical properties		
pH	-	9.8
CEC	cmolc kg^{-1}	101
Carbon[1] (C)	g kg^{-1}	778.0
Total nitrogen (N)	g kg^{-1}	9.1
C/N	-	85.5
Aluminum (Al)	mg kg^{-1}	268
Arsenic (As)	mg kg^{-1}	0.005
Beryllium (Be)	mg kg^{-1}	0.001
Cadmium (Cd)	mg kg^{-1}	0.001
Calcium (Ca)	g kg^{-1}	25.0
Chrome (Cr)	mg kg^{-1}	0.002
Cobalt (Co)	mg kg^{-1}	0.002
Copper (Cu)	mg kg^{-1}	97
Iron (Fe)	mg kg^{-1}	333
Magnesium (Mg)	g kg^{-1}	28.7
Manganese (Mn)	mg kg^{-1}	84
Molybdenum (Mo)	mg kg^{-1}	2
Phosphorus (P)	g kg^{-1}	23.3
Potassium (K)	g kg^{-1}	13.9
Sodium (Na)	g kg^{-1}	11.9
Sulphur (S)	mg kg^{-1}	481
Zinc (Zn)	mg kg^{-1}	104

[1]Source: data from Baronti et al., 2014 (with permission)

Table 8.2. pH, electrical conductivity (EC), total C, H, N concentration and KCl extractable NO_3^--N and NH_4^+-N of different rates of aged as compared with fresh biochar fragments

Biochar	pH	EC µS	C g 100 g^{-1}	H g 100 g^{-1}	N g 100 g^{-1}	NO_3^--N mg kg^{-1}	NH_4^+-N mg kg^{-1}
Fresh	9.97a	903.5a	77.6a	1.41	0.23c	5.51b	132.3b
Aged 5 t ha^{-1}	7.81b	129.8b	66.7b	1.48	0.92a	82.5a	248.8a
Aged 15 t ha^{-1}	8.09b	144.8b	66.3b	1.40	0.73b	69.2a	230.9a
Aged 30 t ha^{-1}	8.08b	158.2b	66.1b	1.21	0.97a	83.4a	342.7a
Significance	***	***	*	ns	***	***	**

ns, *, ** and *** = effect not significant or significant at P <0.05, P <0.01 and P <0.001, respectively. In the same column, means followed by the same letter are not statistically different (P <0.05, SNK Test)

Table 8.3. Elemental composition (atomic concentration - at%) of aged (4-year in field conditions at 30t ha^{-1}) biochar surface (S1) and 3 depth (L2, L3 and L4) compared with fresh biochar as determined by XPS

	C	N				O	Al	Si
AGEING		**S1**	**L2**	**L3**	**L4**			
Fresh	91.6	1.2	0.89	0.85	0.76	6.7	0.29	0.47
Aged	55.3	3.15	1.40	1.15	1.13	32.7	3.73	6.50
Significance	***	**2SEM=0.50**				***	***	***
DEPTH								
S1	68.74					24.1	1.53	3.47
L2	74.03					19.0	2.15	3.69
L3	75.01					18.2	2.25	3.49
L4	76					17.6	2.12	3.31
Significance	**ns**					**ns**	**ns**	**ns**
Ageing *Depth	**ns**			**		**ns**	**ns**	**ns**

ns, ** and *** = effect not significant or significant at P <0.01 and P <0.001, respectively. Interaction between biochar and layer significant at P <0.01. Values differing by $\geq$ 2 SEM are statistically different.
Estimated depth layers: S1 (0-5 nm), L2 (5-10nm), L3 (15-20nm), L4 (30-35nm)

Table 8.4. Atomic concentration (at%) of aged (4-year in field conditions at 30t ha^{-1}) biochar surface (S1) and 3 depth (L5, L6 and L7) compared with fresh biochar as determined by XPS

	C				N				O	Al	Si
AGEING	**S1** (0-5 nm)	**L5** (35-40 nm)	**L6** (70-75 nm)	**L7** (105-110 nm)	**S1** (0-5 nm)	**L5** (35-40 nm)	**L6** (70-75 nm)	**L7** (105-110 nm)			
Fresh	79.0	90.2	91.0	91.2	1.02	0.82	0.76	0.80	10.3	0.64	0.33
Aged	50.4	52.8	65.5	69.2	3.81	2.14	1.18	1.13	30.1	3.51	5.93
Significance	**2SEM=4.82**				**2SEM=0.81**				***	***	***
DEPTH											
S1									28.0a	1.69	3.16
L5									21.2b	2.33	3.51
L6									16.4c	2.03	2.89
L7									15. c	2.23	2.98
Significance									***	ns	ns
Ageing*Depth		*				*			ns	ns	ns

ns, * and *** = effect not significant or significant at P <0.05 and P <0.001, respectively. In the same column, means followed by the same letter are not statistically different (P <0.05, SNK Test). Interaction between biochar and depth significant at P <0.05. Values differing by $\geq$ 2 SEM are statistically different.
Estimated depth layers: S1 (0-5 nm), L5 (35-40nm), L6 (70-75nm), L7 (105-110nm)

Table 8.5. C1s bonding state and relative atomic percentage of aged (4-year in field conditions at 30t ha^{-1}) biochar surface (S1) and 3 depth (L5, L3 and L7) compared with the fresh biochar as determined by XPS

	Binding Energy (eV) (avg ± std dev)															
	-C-C/-CH/-C=C				**-C-O**				**-C=O**				**-COOH**			
	***284.79*±0.05**	***284.76*±0.06**	***284.75*±0.04**	***284.75*±0.05**	***286.14*±0.46**	***285.96*±0.29**	***285.91*±0.21**	***285.85*±0.12**	***287.53*±0.5**	***286.91*±1.28**	***287.16*±0.61**	***287.19*±0.22**	***288.76*±0.39**	**288.87±0.32**	***288.61*±0.45**	***288.73*±0.35**
AGEING	**S1**	**L5**	**L6**	**L7**	**S1**	**L5**	**L6**	**L7**	**S1**	**L5**	**L6**	**L7**	**S1**	**L5**	**L6**	**L7**
Fresh	75.5	67.2	65.8	65.2	13.2	23.9	24.7	25.2	5.1	5.1	5.6	5.6	6.1	3.8	3.9	4.0
Aged	51.9	79.8	78.9	73.7	27.4	15.4	16.4	18.8	12.5	3.0	3.1	4.7	8.15	1.7	1.5	2.8
Significance		**2SEM=8.79**				**2SEM=7.35**				**2SEM=1.77**				**2SEM=1.29**		
Ageing*Depth		******				*****				*******				*****		

*, ** and *** = Interaction between ageing and depth significant at P <0.05, P <0,01 and P <0.001, respectively. Values differing by ≥ 2 SEM are statistically different.
Estimated depth layers: S1 (0-5 nm), L5 (35-40nm), L6 (70-75nm), L7 (105-110nm)

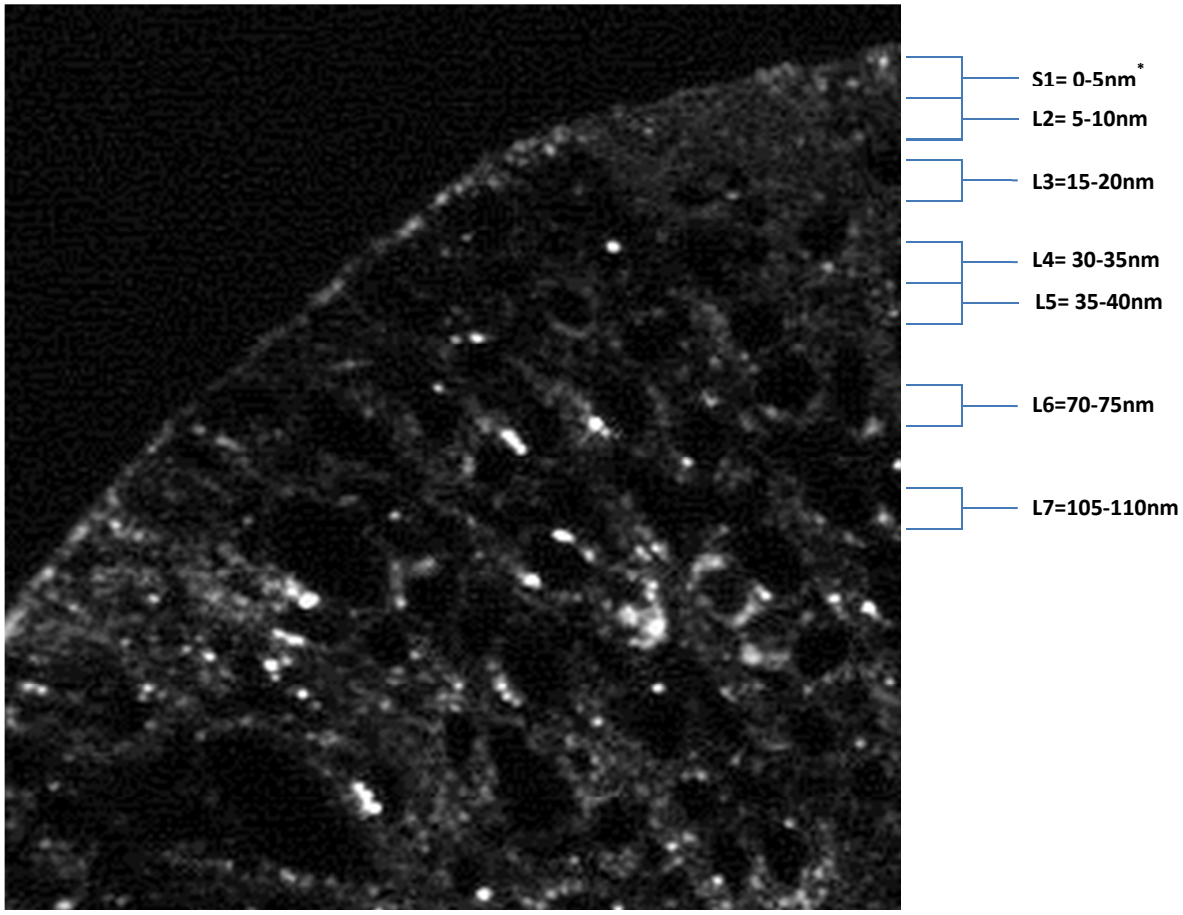

Figure 8.1. Example of biochar profiles scanned by X-ray photoelectron spectroscopy (XPS). Magnification was obtained by a Zeiss SteREO Discovery.V20 microscope

Depths are not strictly to scale

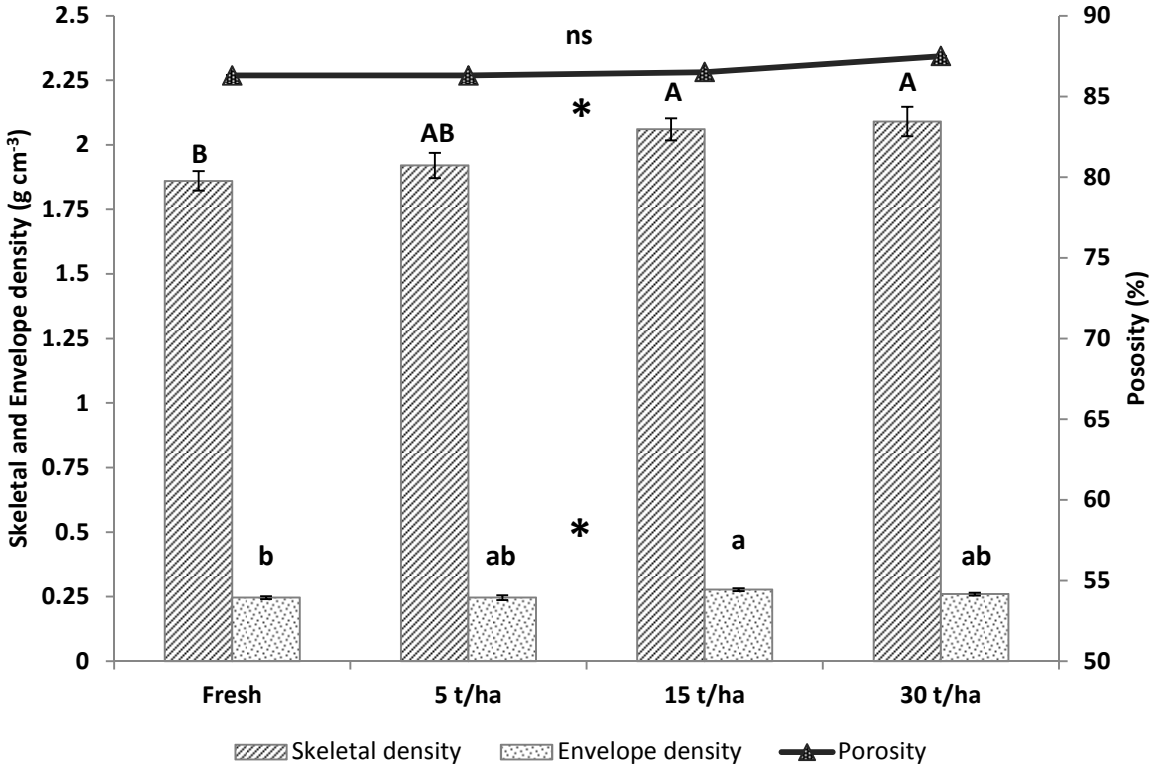

Figure 8.2. Effect of environmental exposure (4 years in field conditions) on density (skeletal and envelope) and porosity of biochar fragments (avg. ± SE n=5) applied at different rates as compared with fresh biochar

ns and * = effect of biochar ageing and rate not significant or significant at $P \leq 0.05$. Bars with the same letter are not statistically different ($P < 0.05$) according to the Student-Neuman-Keuls (SNK) test.

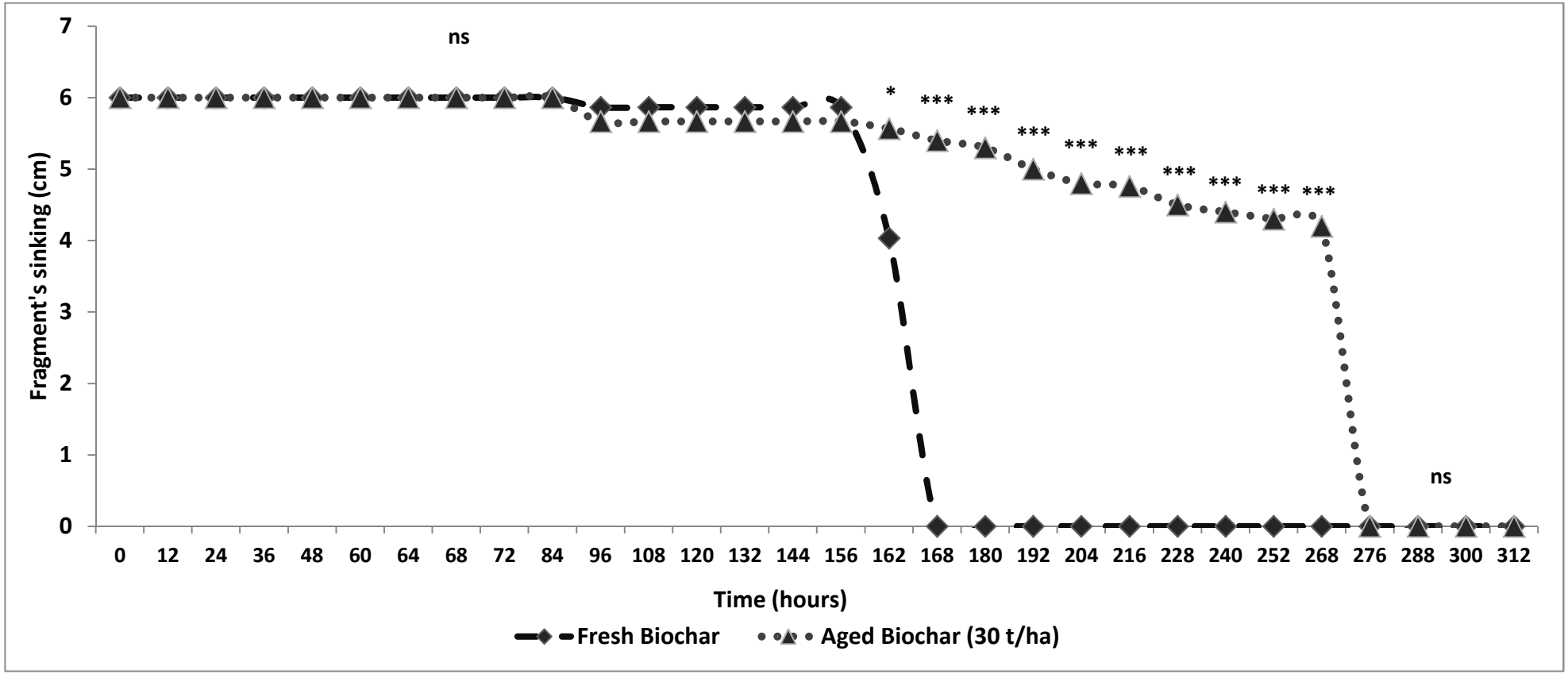

Figure 8.3. Sinking dynamics of fresh vs. aged (4 years in field conditions at the rate of 30 t ha^{-1}) biochar fragments (n=3)

ns, * and *** = effect not significant or significant at $P \leq 0.05$ and 0.001, respectively.

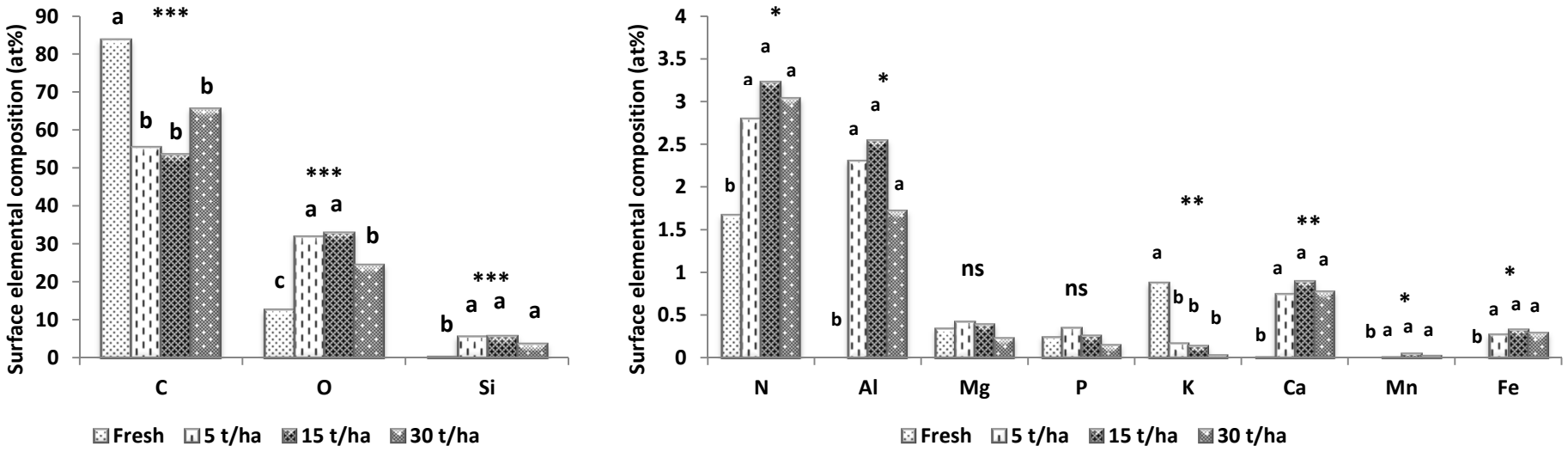

Figure 8.4. Atomic percentage surface elemental composition (XPS) of aged (4-year) biochar applied at different rates as compared with fresh biochar

ns, *, ** and *** = effect of biochar ageing and rate not significant and significant at P $\leq$ 0.05, 0.01 and 0.001, respectively. Within each element, bars with the same letter are not statistically different (P <0.05), according to the Student-Neuman-Keuls (SNK) test.

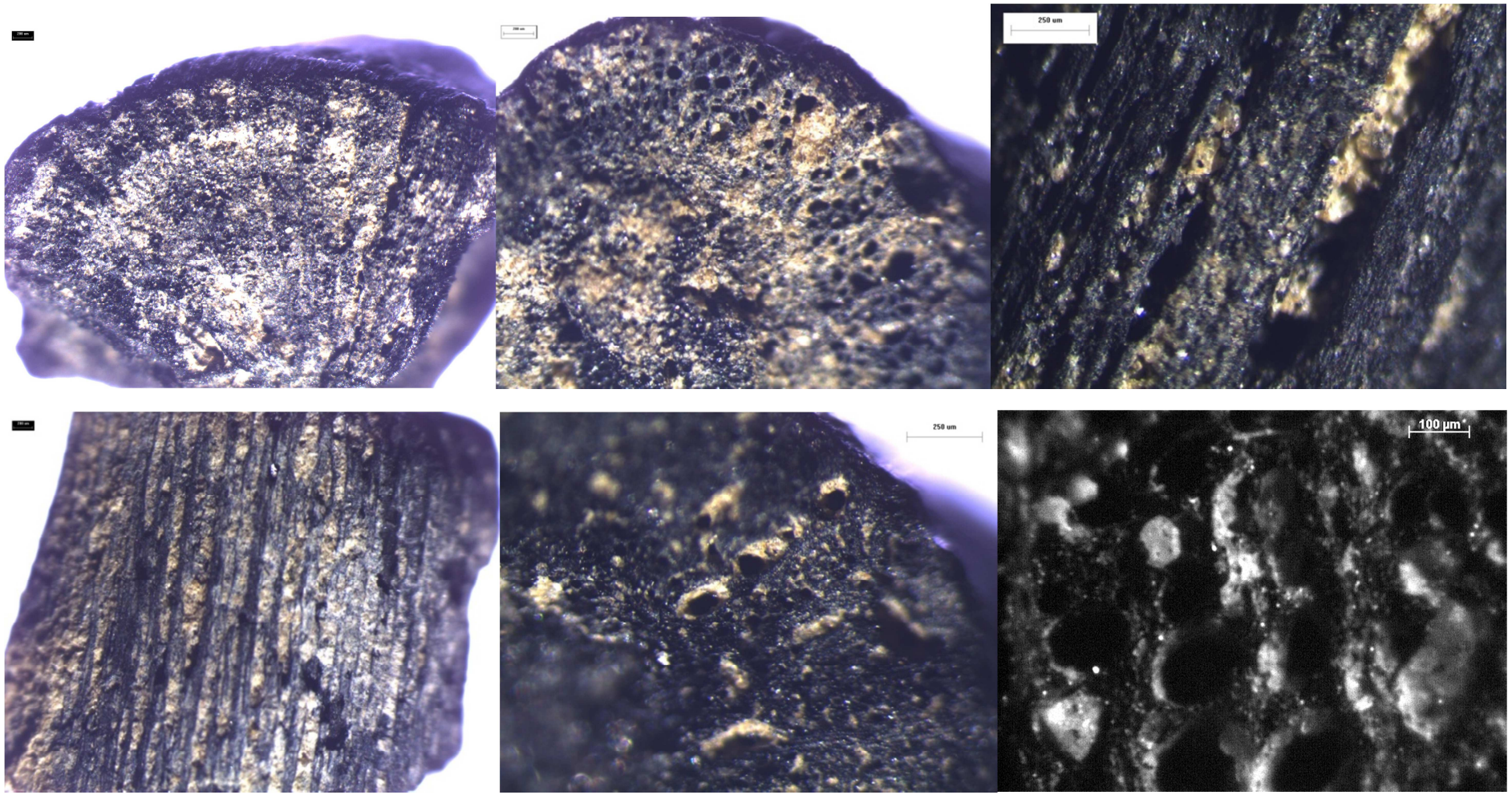

Figure 8.5. Magnification of biochar fragments recovered from a nectarine orchard after 4 years of environmental exposure. Minerals and soil particles are adhering and/or are physically trapped over the entire particle surface. Pores appear partially or totally blocked by soil particles, likely reducing accessibility. Color magnification were obtained by an Olympus SXZ16 microscope coupled with an Olympus digital camera whereas others were obtained by a Zeiss SteREO Discovery.V20 microscope