Influence of Soil Amendments and Soil Properties on Macro–and Micronutrient Availability to Microorganisms and Plants

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Cover: Two experiments included in this thesis; pots in the background and growth boxes in the foreground.
(photo: Atefeh Ramezanian Bajgiran, edited by Amir Ramezanian Bajgiran)

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Abstract

Utilising by-products from industrial and domestic activities and from bioenergy production is one of the new ways of recovering and re-using nutrient resources in agriculture. However, these by-products can potentially add toxic elements or alter soil properties in ways that harm the soil and related environments. This thesis investigated the efficacy and potential adverse effects of using organic (biogas digestate, pot ale) and inorganic (rockdust, wood ash) by-products as amendments on the supply of nutrients to crops (wheat, mixed perennial ryegrass and red clover) and the impact on community-level physiological and genetic profiles of soil microorganisms. The influence of the inherent soil macro –and micronutrient concentration relative to a range of environmental variables was also investigated to explain the variation in physiological profiles of the microbial communities and the genotypic variation in *Rhizobium/Agrobacterium* in a landscape-scale study of pasture and arable soils.

The nutrient status of soils proved to be an important factor for the efficacy of amendment application. The by-products studied generally enhanced crop biomass and the content of some macronutrients and micronutrients in soils and plants when applied to nutrient-poor soils. The concentration of potentially toxic elements (Cd, Pb) was not increased in soils or plants due to amendment application. The botanical composition of mixed ryegrass-red clover stands was also affected by amendments, with biogas digestate, rockdust and wood ash producing more clover than grass. Soil microorganisms were largely unaffected by these amendments. However, the soil microbial community composition was altered by increasing the availability of nutrients through a fully-fertilised treatment. The landscape study showed that *aqua regia*-extractable manganese and rainfall, respectively, were the main factors explaining the variation in microbial physiological profiles and *Rhizobium/Agrobacterium* genotypes.

Keywords: biogas digestate, CLPP, pot ale, red clover, rhizobia, rockdust, ryegrass, T-RFLP, wood ash.

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Dedication

To my mother and father

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ramezanian, A., Dahlin, A.S., Campbell, C.D., Hillier, S., Mannerstedt-Fogelfors, B. and Öborn, I. (2012) Addition of a volcanic rockdust to soils has no observable effects on plant yield and nutrient status or on soil microbial activity. *Plant and Soil*, doi: 10.1007/s11104-012-1474-2.

II Ramezanian, A., Dahlin, A.S., Campbell, C.D., Hillier, S. and Öborn, I. Assessing biogas digestate, pot ale, wood ash and rockdust as soil amendments: Effects on available macro- and micronutrients and microbial community composition. *Manuscript*

III Dahlin, A.S., Ramezanian, A., Campbell, C.D., Hillier, S. and Öborn, I. Recycling of by-products to grass-clover mixtures affects crop growth and quality. *Submitted*

IV Ramezanian, A., Campbell, C.D., Hillier, S., Dahlin, A.S. and Öborn, I. Effect of soil chemical and mineralogical properties on microbial community composition in arable and pasture soils -a landscape study. *Manuscript*

Papers I is reproduced with the permission of the publisher.

The contribution of Atefeh Ramezanian Bajgiran to the papers included in this thesis was as follows:

I Performed the experiment (outdoor and laboratory work) and data analyses, wrote the paper guided by co-authors.

II Planned and performed the pot experiment (outdoor and laboratory work) together with the second author, carried out data analyses and wrote the paper, guided by co-authors.

III Planned and performed the pot experiment (outdoor and laboratory work) together with the first author, reviewed and commented on the manuscript together with the co-authors

IV Prepared the biological data for the dataset (laboratory work), carried out data analyses, wrote the manuscript, guided by co-authors

Abbreviations

Al	Aluminium
ANOVA	Analysis of variance
As	Arsenic
B	Boron
Ba	Barium
Ca	Calcium
Cd	Cadmium
CLPP	Community-level physiological profiles
Co	Cobalt
Cr	Chromium
Cu	Copper
DCA	Detrended correspondence analysis
Fe	Iron
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Ni	Nickel
NSIS	National Soil Inventory of Scotland
P	Phosphorus
RDA	Redundancy analysis
S	Sulphur
Se	Selenium
SIR	Substrate-induced respiration
Ti	Titanium
T-RFLP	Terminal-restriction fragment length polymorphism
XRD	X-ray diffraction
Zn	Zinc

1 Introduction

1.1 In need of sustainable food and feed

The increasing global population coupled with the challenges of environmental degradation and an increasingly variable climate have created a world-wide need for improved food security (e.g. Beddington *et al.*, 2012; Godfray *et al.*, 2010). While much of this need is in developing countries, there are also issues for developed countries. This has led to demands for local food production to reduce air miles and improve rural employment and wealth creation and for food to be produced more sustainably, with improved nutrient status using fewer inputs. In particular, there has been a significant increase in demand for organic products in recent years. The costs of synthetic fertilisers in economic and environmental terms and issues with their long-term availability have also focused minds on finding alternative ways of optimising the use of inherent and exogenous sources of plant nutrients. Meeting the target of increasing the volume of crop products grown, which are used either directly or indirectly (e.g. animal feed to produce meat and milk), also requires more sustainable nutrient management strategies. Such strategies must be based on better information about site-specific properties and local resources, including knowledge of the mineral-rich soil parent materials and options for using local nutrient (re)cycling, in addition to using improved crop species and crop mixtures that are more efficient in taking up and using nutrients (Watson *et al.*, 2012).

1.2 Macronutrient and micronutrient requirements of plants and microorganisms

Organisms are primarily composed of carbon, hydrogen and oxygen, but a number of other elements are also necessary as components of structural

tissues or as participants in biochemical reactions and are termed nutrient elements. The essential nutrients for plants are divided into two groups, macronutrients (N, P, S, K, Ca, Mg, Cl) and micronutrients (B, Co, Cu, Fe, Mn, Mo, Ni, Zn) (Whitehead, 2000). While macronutrients have huge effects on the yield of crops and are needed in large amounts, micronutrient deficiency can also considerably reduce the yield and nutritional quality of crop products, although they are not required in large amounts (Alloway, 2008). Nutrient deficiency can also occur in humans and animals upon consumption of low-quality foods and feeds. Sillanpää (1990) estimated that in global terms, the area of micronutrient-deficient agricultural soils is 49% in Zn, 31% in B, 15% in Mo, 14% in Cu, 10% in Mn, and 3% in Fe. According to Graham (2008), more than half the human population is deficient in Fe, at least half is deficient in Zn, 25% in I and 20% in Se.

Consequently as part of the global efforts to improve food security there is a specific need to focus on how best to optimise food and feed quality (Graham, 2008). The micronutrient concentrations in crops are dependent on a number of factors related to plant species or soil conditions (e.g. Sinclair & Edwards, 2008), stage of growth and climate effects. The important factors of the plant availability of micronutrients in soil include chemical, physical and biological soil processes that control their speciation and solubility in the soil solution. Soil factors such as pH, carbonate content, texture, organic matter, redox potential, complexing ligands, moisture and temperature are all important. Crop management factors also play a role, particularly crop type and crop sequence, supply of macronutrients, liming and tillage practices. Crop species and cultivars may also have differing ability to take up and concentrate micronutrients (Lindström *et al.*, 2012). In the case of micronutrients, while they are essential in trace amounts, high concentrations in soils may result in toxic effects in plants and animals (Whitehead, 2000). In fact, the borderline between deficiency and toxicity is often narrow (e.g. in the case of B; Gupta *et al.*, 1985). Therefore it is vital to choose nutrient sources that match plant/animal requirements, but to avoid accumulation to toxic concentrations. Microorganisms also require macronutrients and micronutrients and are important in the soil-plant system because they are essential for the turnover and recycling of nutrients. Some microorganisms are important symbionts with plants that help acquire nutrients e.g. by fixation of nitrogen by rhizobia. The health, vitality and growth rate of plants in soil is dependent on soil microorganisms, but the micronutrient requirements of soil organisms are often overlooked.

Rhizobia bacteria are known to require B, Co, Cu, Fe, Mn, Mo, Ni, Se and Zn for their survival as free-living soil saprophytes, as well as their symbiotic

relationship with legumes (O'Hara, 2001; O'Hara *et al.*, 1988). The response of rhizobia to nutrient deficiency varies considerably between genera, species and strains (O'Hara, 2001). Microorganisms also suffer from toxicity effects if there are excessive available micronutrients and may in some cases be more sensitive than plants and animals. For example, the adverse effects of excessive concentrations of micronutrients on legume health and nitrogen fixation have been shown to be due not to direct phytotoxicity of the metals, but to the deleterious impact on soil rhizobia (McGrath, 1994).

1.3 Sources of nutrients in soils

1.3.1 Minerals

The Earth's crust is made up of 95% igneous rocks and 5% sedimentary rocks. Of the latter, about 80% are shales, 15% sandstones and 5% limestone (Thornton, 1981). Soils developed from rocks and their constituent minerals tend to reflect their chemical composition (Table 1 for micronutrients), though pedogenetic processes may modify this relationship. Soils derived from the weathering of coarse-grained materials such as sands and sandstones and from acid igneous rocks such as rhyolites and granites tend to contain smaller amounts of nutritionally essential metals, including Cu, Zn and Co, than those derived from fine-grained sedimentary rocks such as shales, and from basic igneous rocks (He *et al.*, 2005).

Mineral weathering is one of the key processes in the formation of soils and sediments, underpinning the inherent soil fertility status, as both macronutrients and micronutrients are released into the soil solution by weathering over long periods of time (Wilson, 2004; Barker *et al.*, 1998). The nature of the soil parent material is usually the main influence on the amounts of the nutrients, other than N in the soil. The input of nutrients released by weathering depends upon their original content in the parent material and upon the stability of the rocks and minerals in which they are contained. A generalised rock stability ranking for soil formation, from more to less stable, is quartzite, chert > granite, basalt > sandstone, siltstone > dolomite, limestone (Anderson, 1988). Examples of minerals include olivine and anorthite, which are the most susceptible to weathering, whereas quartz, muscovite and K-feldspar are particularly resistant (Wilson, 2004) (Figure 1).

It is generally accepted that there is also a strong biological component in many weathering processes (Balogh-Brunstad *et al.*, 2008; Daghino *et al.*, 2008; Gadd, 2008; van Scholl *et al.*, 2008; Gadd, 2007; Barker *et al.*, 1998). Bioweathering is the erosion, decay and decomposition of rocks and minerals

Table 1. Micronutrients in major rock types (mg kg^{-1})

Rock type	B	Co	Cu	I	Fe(%)	Mn	Mo	Ni	Se	Zn
Magmatic Rocks										
Ultramafic rocks	1-5	100-200	10-40	0.01-0.50	9.4-10.0	850-1500	0.2-0.3	1400-2000	0.02-0.05	40-60
Dunites, peridotites, pyroxenites										
Mafic rocks	5-20	35-50	60-120	0.08-0.50	5.6-8.7	1200-2000	1.0-1.5	130-160	0.01-0.05	80-120
Basalts, gabbros										
Intermediate rocks	9-25	1-10	15-80	0.3-0.5	3.7-5.9	500-1200	0.6-1.0	5-55	0.02-0.05	40-100
Diorites, syenites										
Acid rocks	10-30	1-7	10-30	0.2-0.5	1.4-2.7	350-600	1-2	5-15	0.01-0.05	40-60
Granites, gneisses										
Acid rocks (volcanic)	15-25	15	5-20	0.1-0.5	2.6	600-1200	2	20	0.02-0.05	40-100
Rhyolites, trachytes, dacites										
Sedimentary Rocks										
Argillaceous sediments	120	14-20	40-60	1.0-2.2	3.3-4.7	400-800	2.0-2.6	40-90	0.4-0.6	80-120
Shales	130	11-20	40	2-6	4.3-4.8	500-850	0.7-2.6	50-70	0.6	80-120
Sandstones	30	0.3-10	5-30	0.5-1.5	1.0-3.0	100-500	0.2-0.8	5-20	0.05-0.08	15-30
Limestones, dolomites	20-30	0.1-3.0	2-10	0.5-3.0	0.4-1.0	200-1000	0.16-0.4	7-20	0.03-0.1	10-25

Source: (Kabata-Pendias, 2001).

mediated by living organisms, through biomechanical and biochemical attack on minerals (Gadd, 2007). Physical properties (e.g. porosity) and elemental composition may in turn control the initial establishment, growth and survival of the microbial communities on the host rock. Strong correlations between the proximity of microbial cells to mineral surfaces and increased K release from biotite have been demonstrated through model rhizosphere studies (Barker *et al.*, 1998). There is also some evidence that mineral composition affects the associated microbial communities in different environments (e.g. Boyd *et al.*, 2007; Certini *et al.*, 2004), including soil (Carson *et al.*, 2009; Carson *et al.*, 2007).

Most research has concentrated on the release of macronutrients such as K, Ca and Mg. However, soil is also the primary natural source of micronutrients, but there has been less research on how they are derived from minerals and the consequences of this for organisms living in, or depending on, soil for their nutrients. The studies cited above focus on the relationship between microbial colonisation and the macronutrient constituents of different minerals. However, the micronutrient constituents of minerals may also be influential in this regard.

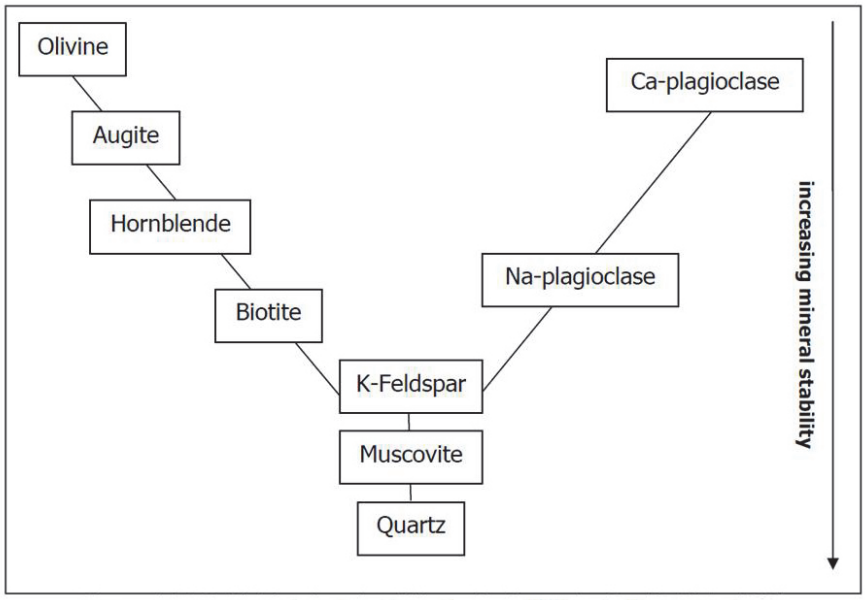

Figure 1. Goldich's minerals-stability series in weathering (Goldich, 1938).

1.3.2 Amendments – inorganic and organic

Utilising by-products from industrial and domestic activities and energy production has gained renewed interest as new ways to recover nutrient and organic matter sources for land application are sought. In addition, the demand for products that meet organic farming criteria adds to an additional dimension to the need to test the efficacy and potential of such by-products as soil amendments and plant nutrient sources. Application of these materials to cropland could affect soil properties, but the effects may not be apparent over short periods. The slow release of these nutrients is responsible for the increase in crop yields in subsequent years, so short-term evaluation of the true agronomic value of these materials as amendments is limited (Diacono & Montemuro, 2010). However, at the same time as enhancing the nutrient status of the soils, these by-products can potentially add toxic elements or alter soil properties in ways that could potentially harm the soil and related environments. Consequently, their efficacy and any associated risks need to be evaluated more carefully. This includes understanding not only the effects on plant growth and nutrient content, but also the wider ecological effects, e.g. on the soil biota responsible for many soil functions (Arthurson, 2009). Soil microorganisms are among the most studied indicators for evaluating soil quality and are useful in part because they respond rapidly to stressors (Ritz *et al.*, 2009; Schloter *et al.*, 2003).

Animal manures have traditionally been an organic soil amendment and nutrient source recycled into crop production on livestock and mixed farms. However, on arable farms and in areas dominated by arable production, there is a need to find other ways to close nutrient cycles and get nutrients back into agriculture. The increasing demand for renewable energy is also leading to increasing amounts of by-products such as biogas digestate and biomass ash, so there is an increasing range of potential materials with varying characteristics for use as soil amendments. Other traditional industries are also looking to recycle by-products. Rock quarries produce potentially useful by-products such as rockdust, which provides freshly fractured unweathered mineral surfaces that are potentially able to supply plants with essential macronutrients and micronutrients (Manning, 2010; Van Straaten, 2006). Pot ale, a liquid by-product from whisky distilleries, in addition to containing a range of nutrients such as P, N, and K, contains Cu due to the use of copper vessels for distillation (Bucknall *et al.*, 1979). Many of these by-products vary geographically, by process and seasonally, creating a wide variation in properties that must be matched to local soil conditions. There is thus a need for more information and understanding of their interactions with soil and crops.

This thesis examined the effects of different soil amendments on a range of soil types. It specifically investigated their efficacy and their interactions with soil microorganisms and with crops, with the emphasis on the influence of micronutrients and minerals.

2 Aims

The overall aim of this thesis was to investigate how soil microbial communities are influenced by the macronutrient and micronutrient status of the soil. The study included nutrients either added to soils by organic and inorganic amendments, or originating from the inherent mineralogy of the soil. In addition, the efficacy of soil amendments in improving crop growth and plant nutrient status of wheat grain and grass-clover mixtures was investigated. The hypotheses and specific questions posed were:

1. Rockdust as a slow-release source of nutrient elements improves soil nutrient concentrations and availability for microbes and plants (Paper I)
   - How does rockdust application affect soil microbial communities (as a result of alteration of soil properties and/or the mineralogy of the soils)?
   - What is the short (1 year) and longer term (3 years) response to rockdust application in terms of crop growth, quality and chemical composition of the soils?
   - Is there any risk regarding the potential toxic elements contained in the rockdust for plants or microbes?

2. Adding selected organic and inorganic soil amendments to nutrient-poor soils improves the availability of elements in these soils for microbes and plants (Papers II, III)
   - Is rockdust more effective when applied to nutrient-poor soils than to the soils studied in Paper I?
   - How are the botanical composition and concentrations of elements affected by different amendments in mixed grass-clover stands?
   - Is there any alteration in the microbial community composition of the soils in terms of physiological and genetic (*Rhizobium/Agrobacterium)* profiles after applying soil amendments?

3. Soil mineralogy and geochemistry (element concentrations) are able to explain the variations in microbial community composition of agricultural soils on a landscape scale (Paper IV)

- What are the main drivers of soil microbial communities in terms of physiological and genetic (*Rhizobium/Agrobacterium)* profiles?
- Is agricultural intensity (permanent pasture, rotational pasture, arable) reflected in the microbial data?
- Do other environmental variables/soil properties play a role and how important are they?

3 Materials and methods

3.1 Overview of experimental approaches

In order to test the effects of mineral soil amendment on nutrient concentrations in soil and plants and the possible effect on soil microbial communities, soils were treated with rockdust at the start of a three-year outdoor experiment in 50-litre containers. Rockdust is claimed to be a slow-release nutrient source in the soil, so this study tested its efficacy as a soil amendment in the years after application. The soils used were from two agricultural fields with different textures (clay and loamy sand), in addition to a commercial garden peat (Paper I).

A pot experiment was set up to test the effect of biogas digestate, pot ale and wood ash as organic and inorganic soil amendments on the availability of elements (nutrients and potentially toxics) in soils and the growth, botanical and chemical composition of a mixture of two forage species. In addition, the possible effects on soil microbial communities were studied, in particular on *Rhizobium/Agrobacterium* genotypes. Since the soils in Paper I, where rockdust was tested, had reasonable nutrient concentrations, rockdust was also added in the following experiments, in which two nutrient-poor soils were used (Papers II and III).

In order to investigate the major environmental drivers of soil microbial communities over a wider range of soil types (including soil element status and mineralogy), the physiological profiles of the microbial communities and DNA fingerprints of *Rhizobium/Agrobacterium* genotypes were measured in a landscape study using soils from the National Soil Inventory of Scotland (NSIS) (Paper IV).

3.2 Soils and amendments used in Papers I-III

The soils used in Papers I-III were collected from different locations in Sweden. The post-glacial clay and loamy sand used in Paper I were collected from near Uppsala (59°49'N, 17°39'E). The nutrient-poor soils used in Papers II and III were collected from Rådde (57°36'N, 13°15'E) and Hollsby (59°48'N, 13°31'E) in western Sweden. The Rådde soil was a till with sandy loam texture developed from gneissic and granitic parent material, and the Hollsby soil was a post-glacial silt loam originating from mainly granitic and sandstone bedrock (Geological Survey of Sweden). The horticultural peat used in Paper I was bought from a commercial garden centre. The characteristics of the soils are summarised in Table 2. Additional experimental soil data are presented in Papers I-III.

The amendments used in Papers II and III were biogas digestate obtained from a biogas plant in Sweden fed source-separated household waste and grass silage; a composite pot ale from several whisky distilleries in Scotland; commercially available volcanic rockdust from the SEER Centre in Perthshire, Scotland (also in Paper I); and wood ash from bottom ash produced following the combustion of mixed deciduous wood on a farm in central Sweden. Chemical characteristics of these amendments are presented in Table 3.

Table 2. *Summary of physical, chemical and mineralogical properties of the soils used in Paper I (Clay, Loamy sand, Peat) and in Papers II and III (Hollsby and Rådde). All units in % except for pH_{H2O} and C:N ratio. The mineralogical composition is given as % of mineral material, i.e. the sum of the minerals is 100%*

	Clay	Loamy sand	Peat[1]	Hollsby	Rådde
Clay	63	9	----	4	8
Silt	31	9	----	69	31
Sand	6	82	----	27	61
pH_{H2O}	6.9	6.3	6.8	5.4	5.5
C_{tot}	2.1	1.3	29	2.2	3.5
N_{tot}	0.23	0.09	1.5	0.19	0.28
C:N ratio	9.1	14	25	12	13
Quartz	21	41	42	49	47
K-feldspar	12	16	13	15.6	14
Plagioclase	17	25	25	17.6	18
Amphibole	3.1	3.2	3.3	1.7	3.9
Goethite	0	0	0	1	0.9
Hematite	0	0	0	0.4	0.2
Clay minerals[2]	44	14	12	7.3	7.6

[1] Low-humified sphagnum 75%v/v, highly-humified sphagnum 20%v/v, sand 5%v/v at the time of purchase.
[2] Clay minerals include illite, kaolinite, and trioctahedral minerals.

Table 3. *Chemical characteristics of the amendments used in Papers I-III (dry weight basis)*

		Amendments			
Parameter	Unit	Biogas digestate	Pot ale	Rockdust	Wood ash
Liming effect	%CaO	6.2	-4	1.9	5100
C_{tot}	%	42	47	0.005	1.1
N_{tot}	%	2.4	5.8	0.097	0.01
Ca_{tot}	g kg^{-1}	48	1.7	13	324
Fe_{tot}	g kg^{-1}	3.7	0.05	31	10
K_{tot}	g kg^{-1}	12	32	2.6	69
Mg_{tot}	g kg^{-1}	6.2	6.0	17	40
P_{tot}	g kg^{-1}	8.3	15	1.2	21
S_{tot}	g kg^{-1}	3.1	4.2	0.09	0.82
Cd_{tot}	mg kg^{-1}	0.10	0.02	0.04	0.27
Co_{tot}	mg kg^{-1}	0.89	0.07	12	21
Cr_{tot}	mg kg^{-1}	13	0.13	12	74
Cu_{tot}	mg kg^{-1}	29	177	7.3	118
Mn_{tot}	mg kg^{-1}	215	16	375	7810
Mo_{tot}	mg kg^{-1}	1.9	0.45	0.20	<6
Ni_{tot}	mg kg^{-1}	5.6	0.41	9.7	121
Pb_{tot}	mg kg^{-1}	9.1	2.9	2.5	2.4
Zn_{tot}	mg kg^{-1}	76	21	46	182

3.3 Experimental set-up, treatments and sampling in different experiments

3.3.1 Effect of rockdust on soils, plants and microbes (Paper I)

The three different soil types (i.e. clay, loamy sand and garden peat) were mixed with volcanic rockdust at the highest (5 kg m^{-2}) and lowest (0.5 kg m^{-2}) application rates recommended by the manufacturer. As a control treatment, quartz sand that was assumed to be inert was applied to the soils at a rate of the 5 kg m^{-2}. Plastic growth boxes (36×55×25 cm, 50-L) were filled with the appropriate treatments in a randomised block design with four replicates and placed outdoors in a netted area. The boxes were planted with one of two cultivars of spring wheat, *Triticum aestivum* L. (cv. Ölandsvete, an old Swedish cultivar not commercially bred, and cv. Triso, a modern Swedish cultivar) in year 1 (2007) and left fallow in year 2. Half the boxes from year 1 (all with cv. Ölandsvete) were sown with mixed stands of perennial ryegrass (*Lolium perenne* L., cv. Helmer) and red clover (*Trifolium pratense* L., cv.

Nancy) in year 3 (2009) and fertilised with ammonium nitrate (equivalent to 30 kg N ha^{-1}) twice during the growing season of year 3.

Grain samples from the two wheat cultivars and shoot biomass samples from the forage species were dried at 50°C, weighed and sent to the laboratory for chemical analyses. Soil samples were taken from each growth box after harvesting of the forage species at the end of the growing season in year 3. These soil samples were subdivided and 1) air-dried and sieved to <2 mm using a plastic sieve for chemical analyses; 2) air-dried for mineralogical analyses, and 3) stored fresh at +4° C for CLPP analysis.

3.3.2 Effect of organic and inorganic amendments on soil, plants and microbes in nutrient-poor soils (Paper II, III)

Biogas digestate, pot ale, rockdust and wood ash as soil amendments were compared with an unamended control and a fully fertilised treatment applied to the two nutrient-poor soils from Hollsby and Rådde. At establishment, 7-L plastic pots (22 cm inner diameter, 25 cm depth) were filled with fresh soil and mixed with the respective amendment in four replicates in late July 2009 (year 0, the establishment year). Pots were then sown with a mixed stand of perennial ryegrass (*L. perenne* L., cv. Helmer) and red clover (*T. pratense* L., cv. Nancy). The pots were kept in an open netted yard in a completely randomised design. Plants were not cut during year 0, and pots were placed in a cold room (-1°C to +1°C) for over-wintering from late November and returned to the outdoor yard in April. Crops were cut three times in year 1 and twice in year 2. Soils were sampled at the end of the growing season in both years 1 and 2. Collected soil samples were homogenised and divided into three plastic bags which were then either: a) air-dried and sieved to <2 mm using a plastic sieve for chemical analyses, b) stored fresh at +4°C for CLPP or c) frozen at -80°C for DNA extraction for T-RFLP analysis.

3.3.3 Landscape study investigating the drivers of soil microbial communities (Paper IV)

The soil samples used in Paper IV were from the national soils inventory of Scotland conducted in 2007-2009, known as NSIS 2. Soil samples from the top Ap horizon (0-15 cm) were collected from sites across Scotland using a 20×20 km^2 sampling grid. Each site included a central profile pit from which a full description of soil characteristics to bedrock (or to minimum depth of 75 cm if bedrock was not visible) was collected. In addition, 4 replicates were taken at random orientations around the central pit at distances of 4, 8, 16 and 20 m, but only from the dominant uppermost horizon. In Paper IV, a total of 220 samples were chosen from the NSIS 2 database, including pasture and arable land uses.

Biological analyses (CLPP and T-RFLP) were performed on the samples. Data on soil chemical, physical and mineralogical properties and on climate were obtained from the National Soil Inventory of Scotland for use in statistical analysis to explain the variation in the measured biological properties. The environmental variables were grouped into three sets, namely soil physicochemical properties ('Physi-chem') including clay, silt, sand, soil dry weight, pH_{H2O}, C and N, C:N, *aqua regia*-extracted Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Sr, Ti and Zn; soil quantitative mineralogical composition ('Minerals') including quartz, plagioclase, K-feldspar, dolomite, iron-rich minerals, clay minerals and organic compounds, and meteorological data ('Climate') including mean temperature and rainfall.

3.4 Analyses of soils and amendments

3.4.1 Chemical analyses

For Paper I, pseudo-total concentrations of macroelements and microelements in the soil samples and rockdust were measured using nitric acid/hydrogen peroxide extraction. For Papers II and III, total concentrations of Cd, Co, Cr, Cu, Mo, Ni, Pb, S and Zn were analysed in soil samples (Rådde and Hollsby) wood ash and rockdust after digestion (concentrated HNO_3 + 2 ml HCl + 2 ml HF) using ICP-SFMS. Samples were also fused with lithium metaborate, then dissolved in HNO_3 and Ca, Fe, K, Mg, Mn, Na and P were measured using ICP-AES. Biogas digestate and pot ale were digested in HNO_3 and HF and all elements determined using ICP-SFMS. EDTA-extractable elements in the soil samples (Papers I-III) were measured using the method described by Streck & Richter (1997) with some modifications, so that Na-EDTA was used and the extracts were analysed by ICP-MS.

3.4.2 Biological analyses

(a) Community-level physiological profiling (CLPP)

The flush of carbon dioxide after the addition of different carbon sources (namely arginine, L-alanine, α-ketoglutaric acid, L-arabinose, citric acid, L-cystein, D-fructose, D-galactose, γ-amino butyric acid, D-glucose, L-lysine, L-malic acid, N-acetyl-glucosamine, oxalic acid and trehalose) was determined by the MicroResp^{TM} method (Campbell *et al*., 2003). Soil samples were sieved to <2 mm, adjusted to 30-40% of water-holding capacity (WHC) and used to fill 96-well microtitre deep-well plates with a capacity of 1.2 ml (Thermo LifeScience, Basingstoke, United Kingdom). After 3-4 days of pre-incubation

at 25°C, freshly prepared solutions of C sources were added to the soil in deep-well plates. Deionised water was used as a control. The CO_2 released from the substrate was trapped in a detection gel cast plate in the 96-well microplate cover. The detection plates were prepared using the indicator cresol red (12.5 ppm, wt/wt), potassium chloride (150 mM), and sodium bicarbonate (2.5 mM) set in 1% purified agar and were read in a microtitre plate reader (VMAX; Molecular Devices, Wokingham, UK) at 570 nm immediately before and after 6 hours of incubation at 25°C. SIR data were calculated by subtracting the values of the CO_2 respired in the control (deionised water) from the respiration after addition of C sources (Campbell *et al*., 2003).

(b) Terminal-restriction fragment length polymorphism (T-RFLP)

DNA was extracted from 0.5 g soil samples stored at -80°C using the FastDNA SPIN kit (MP Biomedicals LLC.) as described by Berthelet *et al*. (1996). A polymerase chain reaction (PCR) was performed using 1 µL of a DNA template in a 50-µL master mix of 10× NH_4 reaction buffer, dNTPs (20 mM), $MgCl_2$ (50 mM), BSA (20 mg/ml), BIOTAQ™ DNA polymerase, dH_2O and the primer set RHIZ-1244 (CTC GCT GCC CAC TGT CAC) and bac 16S 8F (AGA GTT TGA TCC TGG CTC AG), 5' labelled with the fluorescent dye NED™. PCR products were purified using the ChargeSwitch PCR clean-up kit (Invitrogen, United Kingdom) following the manufacturer's instructions. The amount of DNA in samples was measured using NanoDrop (Thermo Fisher Scientific Inc.) to calculate the aliquots needed for restriction. Aliquots containing approximately 500 ng of purified PCR product were restricted using 2 µL per reaction of Hhal (Promega, United Kingdom). Digestates were incubated on a Dyad Peltier thermal cycler (Bio-Rad Laboratories Inc.) at 37°C for 3 hours, at 95°C for 10 minutes and held at 10°C. Up to 2 µL digested sample was added per well on the sequencing plate. 12 µL Hi-Di formamide (ABI Part No 4311320) and 0.3 µL GeneScan™ 500 LIZ™ Size Standard (ABI Part No 4322682) were added to each sample prior to the run. Samples and a positive control (100% *Rhizobium*) were run on an Applied Biosystems 3130xl Genetic Analyser. T-RFLP profiles from each sample were obtained using GeneMapper v4.0 software (Life Technologies Corporation). Fragments at or close to the limit of detection and/or only found in less than 10% of samples were omitted from the analysis.

3.4.3 Mineralogical analyses (XRD)

For quantitative and qualitative mineralogical analysis, air-dried soil and amendment samples were ground in an agate McCrone mill in ethanol. Random powders were prepared by spray-drying the resulting slurries (Hillier,

1999). XRD patterns for quantitative analyses were measured on spray-dried samples run on a Siemens D5000 (Siemens, Germany) using Co K-alpha radiation selected with a graphite monochromator (Papers I-III), or on a Panalytical Xpert Pro diffractometer (Panalytical, the Netherlands) using Ni-filtered Cu K-alpha radiation (Paper IV). All analyses conducted using a full-pattern fitting method (Omotoso *et al*., 2006). The XRD patterns for qualitative analyses of spray-dried samples were recorded on the Panalytical Xpert Pro diffractometer using Ni-filtered Cu K-alpha radiation (Papers I, II).

3.5 Forage and grain analyses

Forage samples (Papers I, III) were separated into the different species after harvest and dried at 50°C, weighed and milled to a particle size below 1 mm using a cutting mill (Grindomix GM 200, Retsch GmbH) with a titanium knife and a plastic container to avoid microelement contamination (Dahlin *et al*., 2012). Wheat grains were digested as whole grains to avoid contamination from milling devices. Element concentrations in wheat grains (Paper I) and forage biomass (Papers I, III) were analysed by ICP-Optima 7300 DV after wet digestion with HNO_3.

3.6 Statistical analyses

ANOVA with Tukey pair-wise comparisons was used to assess treatment differences in the data obtained in soil, grain and plant chemical analyses and SIR data (Papers I-III) using JMP 9.0.0 (SAS Institute Inc., Cary, NC, USA) and $p<0.05$ as the limit for statistical significance. When needed, data were Box-Cox- (Box & Cox, 1964), ln- or square root-transformed to achieve normal distribution of residuals. Statistical analysis of CLPP data from all added C sources was based on Bray-Curtis dissimilarities (Paper I) and Euclidean distances (Paper II) of SIR data, which were analysed by canonical variate analysis using the CAP programme (Anderson & Willis, 2003). T-RFLP analyses were converted into a binary data table (presence or absence of individual peaks) using MS Excel (Papers II, IV) and analysed by canonical variate analyses based on Bray-Curtis dissimilarities using CAP software (Paper II). Canonical variate analyses (for land use effect on T-RFLP data) were conducted using JMP 9.0.0. (Paper IV). Detrended correspondence analysis (DCA) of both CLPP and T-RFLP data was used to determine the best constrained ordination method fitting the data, after which partial RDA was chosen for variance partitioning, and Monte Carlo permutation testing (9999

permutations at a significant level $p<0.05$ using Bonferroni correction) for forward selection (Lepš & Šmilaur, 2003) (Paper IV).

Table 4. *Summary of the methods used in Papers I-IV*

Material studied	**Specification**	**Method**	**Paper**
Soil analyses	pH_{H2O}	1:5 soil:solution ratio	I
	EC, pH_{H2O},	1:2 soil: solution ratio	II, III,IV
	pH_{CaCl2}	0.01 M $CaCl_2$ in 1:2 soil: solution ratio	II, III
	Soil texture	Pipette method	I, II, III
		Laser diffraction	IV
Elements in soils and amendments			
N & C	Total	High-temperature induction furnace combustion using LECO CN2000	I, II, III
		Automated Dumas combustion procedure using a Flash EA 1112 Elemental Analyser	IV
Other elements except N & C	Total	HNO_3+HCl+HF, analysed by ICP-AES (soils, rockdust, wood ash)	II, III
		HNO_3+HF, analysed by ICP-SFMS (biogas digestate, pot ale)	II, III
	Pseudo-total	Digestion with HNO_3+H_2O_2 , analysed by ICP-SFMS (soils, rockdust)	I
		Aqua regia (soils)	IV
	EDTA	Extracted with Na-EDTA, analysed by ICP-MS ELAN 6100 DRC (soils)	I, II, III
Soil biological analyses	CLPP	Determining SIR using MicroResp method	I, II, IV
	T-RFLP	DNA fingerprinting of *Rhizobium/Agrobacterium*	II, IV
Mineralogical analysis	Soils and amendments	Analysed by X-ray powder diffraction	I, II, III, IV
Plant analyses	Grains and forage	Wet digestion (HNO_3), elements analysed by ICP-Optima 7300 DV	I, III

4 Results

4.1 Outdoor growing experiments

4.1.1 Influence of soil amendments (rockdust, biogas digestate, pot ale and wood ash) on microbial communities

The effect of rockdust, both as a fresh source of nutrient elements and as an alteration to the mineralogy of the soils, was tested on soil microbial communities in two experiments on: (i) Three different soils three years after application (Paper I) and (ii) two nutrient-poor soils one and two years after application (Papers II and III).

The ordination diagram from multivariate analysis of substrate-induced respiration (SIR) showed that regardless of how long ago the rockdust was added (*i.e*. 1, 2 or 3 years) and the nutrient status of the original soil, the greatest difference in CLPP was between the soil types. The discrimination was primarily on CV 1, which explained 81% of the variation (Figure 2). The multivariate analysis of the CLPPs obtained by SIR data for other amendments (*i.e*. biogas digestate, pot ale, wood ash) in addition to control and fully fertilised treatments once again showed that the greatest difference was between the two different soils and was primarily on CV 1, which explained 64% of the variation in soil samples (Figure 3). Soil microbial community composition did not change significantly after application of any of the treatments except in the fully fertilised treatment on both soils, which tended to cluster together separately from other treatments in their soil group (Figure 3). Results for year 2 generally showed the same pattern (data not shown).

The effect of applying rockdust and other amendments to the nutrient-poor soils of Hollsby and Rådde on *Rhizobium/Agrobacterium* genotypes was also investigated using the T-RFLP DNA fingerprinting method. As shown in Figure 4, the greatest discrimination was mostly based on soil type. Rockdust application did not influence the *Rhizobium/Agrobacterium* genotypes

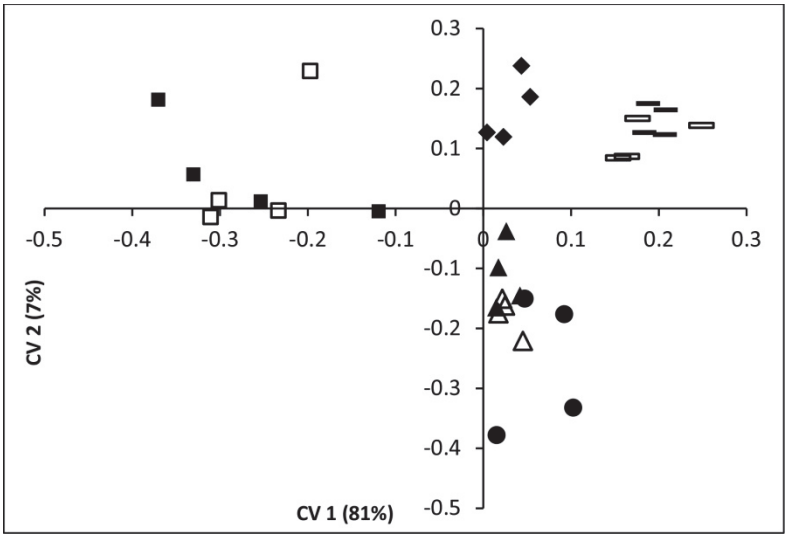

Figure 2. Ordination diagram of the first and second CVs for CLPPs with SIR data after rockdust application to soils (squares=peat, triangles=loamy sand, horizontal bars=clay, diamonds=Hollsby, circles=Rådde), at two different rates (black=5 kg rockdust m^{-2}, white=0.5 kg rockdust m^{-2}).

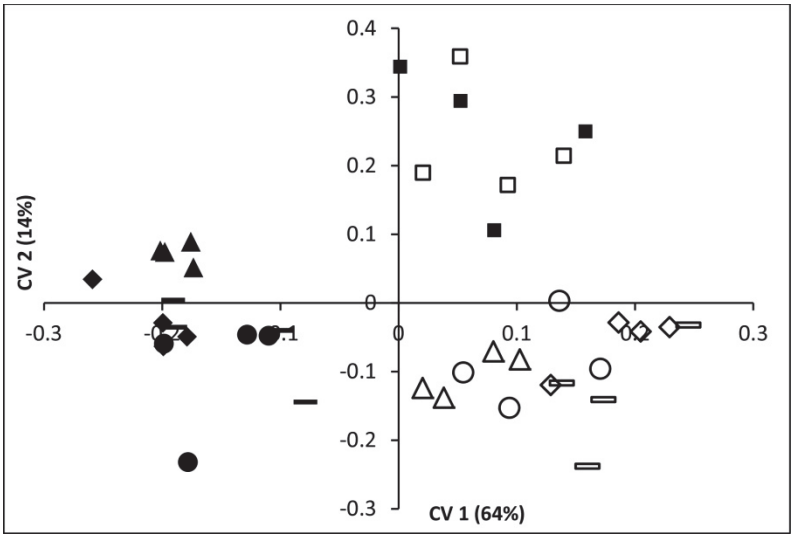

Figure 3. Ordination diagram of first and second CVs for CLPPs with SIR data after application of soil amendment (diamonds=pot ale, circles=biogas digestate, triangles=wood ash, squares=full nutrient solution, horizontal bars=control) to soils (black=Hollsby, white=Rådde).

compared with the unamended control. However, samples from the fully fertilised treatment of both soils were clustered together and significantly discriminated from rockdust and control treatments of either soil. The same pattern generally happened for *Rhizobium/Agrobacterium* genotypes in the soil samples with biogas digestate, pot ale and wood ash application compared with the unamended control and the fully fertilised treatment. Once again, samples from fully fertilised treatments on both soils clustered together, while other amendments and the control clustered according to their relevant soil type (Figure 5). The major discrimination was along the first axis, which was based on different soils. The discrimination along the second axis, which was based on the treatment effect, showed that the fully fertilised treatment and pot ale amendment were significantly different from the other treatments (Figure 5).

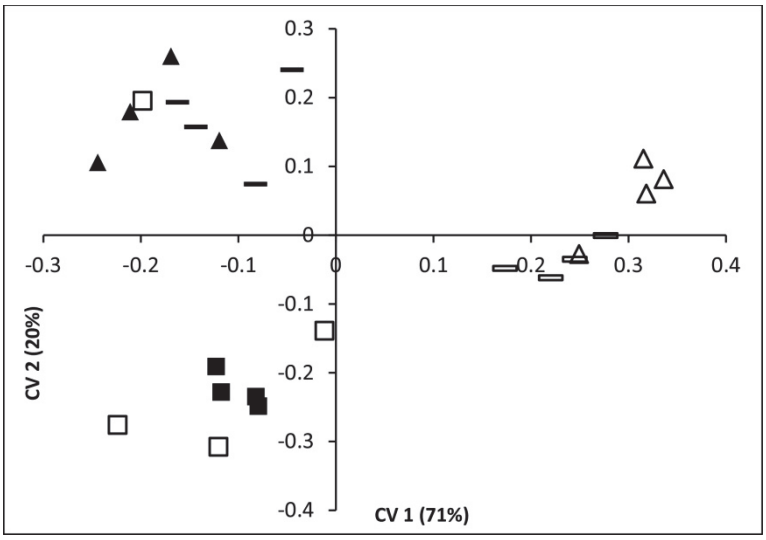

Figure 4. Ordination diagram of first and second CVs of *Rhizobium/Agrobacterium* TRFs after rockdust application to Hollsby and Rådde soils compared with the fully fertilised and unamended control treatments (triangles=rockdust (5 kg m^{-2}), squares=full nutrient solution, horizontal bars=control, black=Hollsby, white=Rådde).

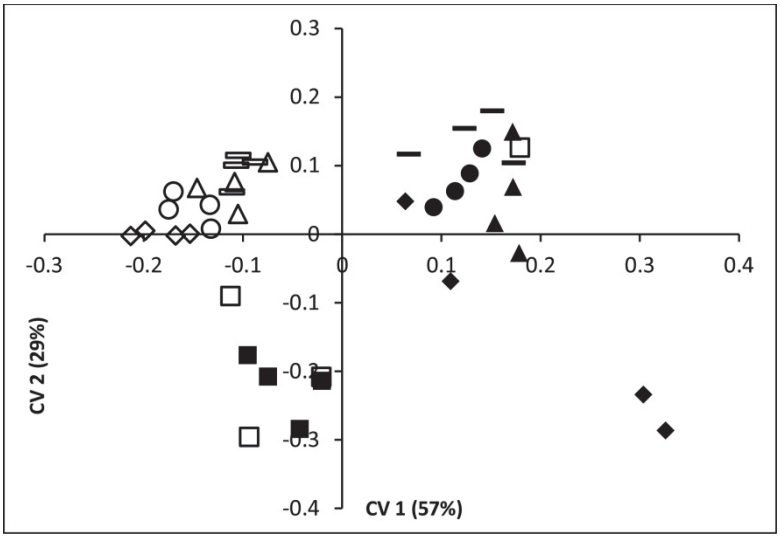

Figure 5. Ordination diagram of first and second CVs of *Rhizobium/Agrobacterium* TRFs after amendments application to Hollsby and Rådde soils (diamonds=pot ale, circles=biogas digestate, triangles=wood ash, squares=full nutrient solution, horizontal bars=unamended control, black=Hollsby, white=Rådde).

4.1.2 Influence of amendments on soil mineralogical and chemical properties and element status

(a) Rockdust

An alteration of the mineralogical composition after applying rockdust to clay, loamy sand and peat soils using XRD was only observed in the peat, which demonstrated an enhanced content of clay minerals and of plagioclase feldspars in the high rate (5 kg m^{-2}) rockdust treatment compared with the low rate (0.5 kg m^{-2}).

Rockdust application did not significantly alter the pH_{H2O}, pH_{CaCl2} or EC of the soils in the two experiments to which it was added. Table 4 shows the pseudo-total concentrations of elements in the three soil types treated with rockdust three years prior sampling and in the rockdust itself. The applied rockdust had lower concentrations of nutrients such as K, Cr, Cu, Mo and Zn compared with all three soils. Furthermore, rockdust had lower concentrations of the potentially toxic non-nutrients Cd and Pb compared with the soils. Rockdust-treated soils generally had a significantly higher pseudo-total concentration of macroelements and microelements compared with the quartz sand-treated controls, but there was no significant difference between the high

Table 4. *Pseudo-total concentrations of macroelements and microelements in original soils and rockdust, and ANOVA of rockdust/quartz sand treatments across the soils (n=4)*

	Macroelements (g kg^{-1} DW)						**Microelements (mg kg^{-1} DW)**								
	Ca	Fe	K	Mg	P	S	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Zn
Clay	7.01	35.20	5.06	9.67	0.74	0.40	0.25	16.4	50.7	28.8	515	0.80	35.8	27.1	122
Peat	26.4	7.75	2.16	7.20	0.92	3.32	0.14	3.39	9.32	26.5	392	4.53	7.85	12.8	65.9
Loamy sand	3.25	11.70	0.90	3.51	0.78	0.16	0.07	4.59	12.0	9.31	264	0.27	6.68	10.7	54.6
Rockdust	5.36	20.20	0.30	12.0	1.14	0.20	0.01	12.4	5.61	8.17	297	<0.2	10.2	1.98	48.5
High rate[1]	6.65^{A}	18.6^{A}	2.19^{A}	5.98^{A}	0.70^{A}	0.36^{A}	0.15^{A}	7.05^{A}	21.8^{A}	16.2^{A}	311^{A}	0.62^{AB}	14.3	14.3^{B}	69.2
Low rate[2]	6.92^{A}	18.7^{A}	2.25^{A}	5.77^{A}	0.69^{A}	0.38^{A}	0.15^{A}	6.71^{A}	22.3^{A}	16.7^{A}	312^{A}	0.67^{A}	14.6	15.1^{A}	65.8
Quartz sand[3]	6.00^{B}	16.7^{B}	2.05^{B}	5.40^{B}	0.61^{B}	0.33^{B}	0.13^{B}	6.21^{B}	20.4^{B}	14.7^{B}	280^{B}	0.56^{B}	14.2	13.4^{C}	63.9
ANOVA	***	***	***	***	***	**	***	***	**	***	***	***	ns	***	ns

*Significance: ns: not significant, ** p< 0.01, ***p<0.001.*

Different letters indicate significant difference as determined by ANOVA

[1] *High application rate of rockdust= 5 kg m^{-2}*

[2] *Low application rate of rockdust= 0.5 kg m^{-2}*

[3] *Quartz sand (5 kg m^{-2})*

and low rate of rockdust application (Table 4). Rockdust application did not have a significant effect on EDTA-extractable concentration of microelements (Co, Cr, Cu, Mn, Mo, Zn) in the three soils (Paper I). However, Cd_{EDTA}, Ni_{EDTA} and Pb_{EDTA} were significantly higher in the soils treated with the low rate of rockdust compared with the other treatments, indicating higher concentrations in the original soils (of EDTA-extractable Cd and Pb) than in the rockdust (Paper I). The Hollsby and Rådde soils used in Papers II and III on which rockdust application was tested had generally lower fertility than the three soils included in Paper I. The total concentration of elements in Hollsby and Rådde soils is shown in Table 5, where the rockdust composition is also given. Rockdust had higher total concentrations of Ca, Fe, Mg, Co, and Ni than the two soils tested. However, EDTA-extractable concentrations were generally not enhanced after rockdust application compared with the unamended control. The only exception was Mg_{EDTA}, which was significantly higher in rockdust-treated soils (both Hollsby and Rådde) than in the unamended controls.

(b) Biogas digestate, pot ale and wood ash

Wood ash-treated soils had significantly higher pH_{H2O}, pH_{CaCl2} and EC than other soils. Biogas digestate and pot ale did not change the pH of the soils significantly compared with the unamended control. Wood ash application significantly increased Ca_{EDTA}, K_{EDTA} and Mg_{EDTA} in both soils. Biogas digestate increased Mg_{EDTA} in Rådde and pot ale increased K_{EDTA}, Mg_{EDTA} and Cu_{EDTA} in both soils compared with the unamended control (Table 6). EDTA-extractable concentration of other elements (*i.e.* P, S, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb and Zn) did not show changes after application of the amendments compared with the unamended control soils.

4.1.3 Influence of amendments on crop biomass, botanical composition and concentrations of elements

(a) Rockdust

One year after application, rockdust did not significantly affect the yield of the two spring wheat cultivars included in the study, nor the concentrations of elements in the wheat grain. The results were consistent for the three different soil types used (clay, loamy sand and garden peat). The application rate of rockdust (high or low) did not make any difference. Biomass yield of the mixed forage species, perennial ryegrass and red clover, planted in the third year after rockdust application on the three soil types, was also not significantly enhanced by the low or high rate of application. However, yield of forage grown in the two nutrient-poor soils (Hollsby and Rådde) was

Table 5. *Total concentrations of macroelements and microelements in Hollsby and Rådde soils and rockdust*

	Macroelements (g kg^{-1} DW)							**Microelements(mg kg^{-1} DW)**								
	Ca	Fe	K	Mg	Na	P	S	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Zn
Hollsby	1.0	16	25	3.0	16.4	0.78	0.33	0.12	2.6	15	6.9	531	0.40	4.4	18	46
Rådde	11	18	21	3.5	15.7	1.2	0.48	0.13	3.8	23	6.5	431	0.85	7.1	19	30
Rockdust	13	31	2.6	17	5.4	1.2	0.094	0.039	12	12	7.3	375	0.20	9.7	2.5	46

improved significantly (by 1.3 fold) by rockdust application compared with the unamended control (Table 7). The botanical composition of the mixed crops, as indicated by the proportion of clover, did not differ between the high and low rate of rockdust application (Paper I), while it increased significantly, by 1.2-fold, in Rådde soil compared with the unamended control (Paper III) (Table 7). Of all the elements tested (*i.e*. C, N, Ca, K, Mg, Na, P, S, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn), applying rockdust to the clay, loamy sand and peat soils only increased the concentration of Na significantly in the plant tissue of both forage species (Paper I). Rockdust had the same effect of increasing the Na concentration in the plant tissue of mixed crops on the Hollsby and Rådde soils and also increased the Mg concentration on Rådde soil compared with the unamended control (Papers II, III). On the other hand, rockdust application significantly decreased the concentration of P, Cu, Mn and Mo analysed in mixed crops on the Hollsby and Rådde soils compared with the unamended control.

Table 6. *EDTA-extractable elements in soils (dry weight basis) as influenced by amendments (n=4)*

	Ca	K	Mg	Cu
	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Soil×treatments	***	***	***	*
Hollsby				
Unamended	0.99^{c}	20^{defg}	16^{gh}	1.4^{bcde}
Fully fertilised	0.77^{de}	30^{abcd}	40^{a}	1.1^{g}
Biogas digestate	1.08^{bc}	27^{bcdef}	16^{gh}	1.5^{bc}
Pot ale	1.03^{c}	33^{abc}	21^{def}	2.0^{a}
Wood ash	1.21^{ab}	34^{ab}	29^{bc}	1.6^{b}
Rådde				
Unamended	0.55^{f}	15^{gh}	5.3^{k}	1.1^{g}
Fully fertilised	0.82^{d}	29^{bcde}	40^{a}	1.1^{g}
Biogas digestate	0.65^{ef}	20^{fg}	7.2^{j}	1.2^{efg}
Pot ale	0.56^{f}	23^{cdef}	9.4^{i}	1.5^{bcd}
Wood ash	0.81^{de}	28^{bcdef}	18^{fg}	1.2^{defg}

*Significance: *p<0.05, ***p<0.001.*
Different letters indicate significant difference as determined by ANOVA

(b) Biogas digestate, pot ale and wood ash

Biogas digestate, pot ale and wood ash significantly enhanced the biomass yield of the mixed crops compared with the unamended controls, but the yield did not reach the level of the fully fertilised treatment (Table 7). The botanical composition under the different amendments was significantly different, with

the pot ale treatment having less clover than the others. The fully fertilised treatment had the significantly lowest proportion of clover (Table 7). The amendments significantly increased the concentration of the elements in mixed crop tissues (in one or both soils) compared with the unamended control as follows: biogas digestate increased Ca, Ni and Mo, pot ale increased K and P, and wood ash increased K, Mg, and Mo. However, the concentrations of some elements in mixed forage crops decreased after application of amendments as compared with the unamended control: N, P, Ca, Mg, and Cu by pot ale application, Cu by biogas digestate application and N, P, Na, Co, Cu, Mn, Ni and Zn by wood ash application.

Table 7. Biomass yield and botanical composition of the mixed crops

	Biomass yield (g pot^{-1})	Clover proportion
Rockdust high rate	58	0.63^{b}
Rockdust low rate	59	0.63^{b}
Quartz sand	61	0.74^{a}
ANOVA	ns	*
Hollsby		
Unamended	24^{def}	0.68^{ab}
Fully fertilised	60^{a}	0.33^{de}
Biogas digestate	33^{b}	0.70^{ab}
Pot ale	30^{bc}	0.49^{c}
Rockdust	30^{bc}	0.77^{a}
Wood ash	33^{b}	0.79^{a}
Rådde		
Unamended	14^{g}	0.48^{c}
Fully fertilised	62^{a}	0.30^{de}
Biogas digestate	26^{cde}	0.64^{b}
Pot ale	20^{f}	0.34^{d}
Rockdust	20^{f}	0.63^{b}
Wood ash	26^{cde}	0.73^{ab}
ANOVA	***	***

*Significance: ns: not significant, *p<0.05, ***p<0.001.*
Different letters indicate significant difference as determined by ANOVA

4.2 Landscape study investigating the drivers of soil microbial communities

The results from Papers I-III show that in the agricultural soils included in the experiments, the discrimination of soil microbial communities was mainly based on soil type and less on the amendments. In Paper IV, a wider range of soils was included and their inherent mineralogy was mainly tested to determine if there were relationships between the minerals, the concentrations of elements in the soils and the microbial communities. Redundancy analysis (RDA) showed that 79% of the variation in CLPP data and 58% of the variation in T-RFLP data was explained by the environmental variables (Figures 6 and 7). Variance partitioning (partial RDA) suggested that soil physicochemical properties explained the majority of the total variance in both CLPP and T-RFLP data. The forward selection with Monte Carlo permutation test showed that the *aqua regia*-extracted concentrations of elements were the most important properties of soils affecting the physiological profiles of soil microbial communities. Mn_{AQR} was the strongest factor in CLPP data, explaining 44% of the variation, but rainfall explained 29% of the total variation in T-RFLP data. A set of variance partitioning performed within the Physi-chem set revealed that microelements had a much greater effect than macroelements in explaining the variation in both CLPP and T-RFLP data. The results also showed that different land uses involving permanent and rotational pastures and arable land had significantly different patterns of T-RFLP due to *Rhizobium/Agrobacterium* genotypes.

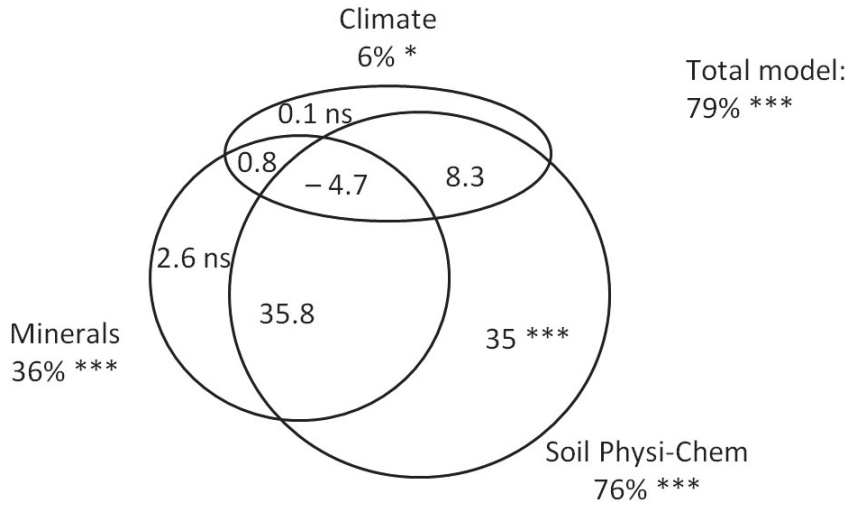

Figure 6. Results of partial RDA with three sets of explanatory variables for CLPP data. A negative shared variance fraction indicates that the joint explanatory effect of those groups of variables is stronger than a sum of their marginal effects. ns: not significant, *p<0.05, ***p<0.001.

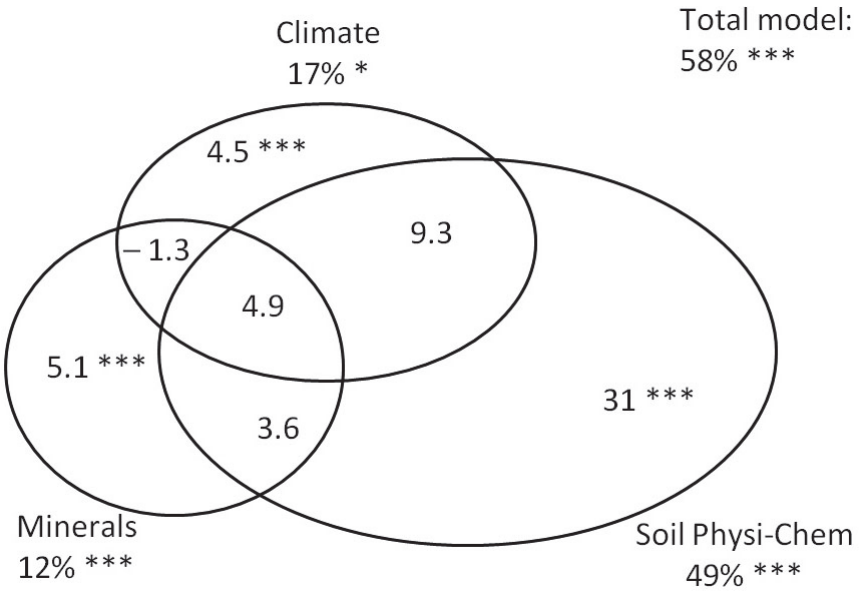

Figure 7. Results of partial RDA with three sets of explanatory variables for T-RFLP data. A negative shared variance fraction indicates that the joint explanatory effect of those groups of variables is stronger than the sum of their marginal effects. *p<0.05, ***p<0.001.

5 Discussion

5.1 Effect of rockdust as slow-release source of nutrients

5.1.1 On soil microorganisms

It was hypothesized that rockdust as a source of nutrients and new mineral surfaces for colonisation might alter microbial responses either through direct effects or indirect effects on plants, but this proved not to be the case. The physiological profiles based on SIR of multiple C sources showed that different soil types included in the experiments, despite producing similar crops, had distinctly different microbial populations. However, rockdust application did not alter the SIR rate or physiological profile of the microbial communities in the soils. Applying rockdust to the soils even at the high rate (6% by weight to the peat soil and 2.7 and 2.5% to the clay and loamy sand soils respectively) did not considerably alter the mineralogy of the soils or significantly affect the microbial community. In contrast, Carson *et al*. (2007) reported that addition of mica, basalt and rock phosphate to soil microcosms induced substantial changes in both bacterial and fungal community composition. In their experiment, the original soil contained 99.8% sand (>99% quartz). However, such a soil was more likely to be deficient in nutrients and adding mica, basalt and rock phosphate could have represented a considerable change in terms of nutrients, leading to a change in microbial communities. However, this is perhaps not the case with agricultural soils.

In conclusion rockdust seems to have little effect on either the physiological or genetic profiles of the microbial community. This is at least a neutral effect in terms of judging the safety of rockdust applications but also points to a negligible microbiological or plant effect of rockdust in the soils tested.

5.1.2 On soil properties and element status

Rock powders are reported to have the potential to improve the nutrient status of soils by releasing nutrients and/or increasing the pH, particularly in acid soils (Winiwarter & Blum, 2008). In the current study, rockdust had a high original alkaline pH, but low liming effect. The relatively high pH measured for the rockdust is most likely an abrasion pH, *i.e*. a phenomenon whereby minerals alter the pH of water when they are ground to a powder. The high (abrasion) pH of the rockdust is probably due to hydrolysis of the minerals it contains. Abrasion pH, which has long been used as a simple test to identify different minerals, varies between 8 and 10 for plagioclase feldspars (Stevens & Carron, 1948), which are the main minerals present in our rockdust according to analysis by XRD. Hence the measured high pH is not accompanied by a significant liming effect and a subsequent increase in the pH of the soils after rockdust application should not be expected as found in both the experiments with rockdust.

British Geological Survey lists the quarried rock from which the rockdust used in this study originates as a pyroxene-andesite (Cameron *et al*., 2010). Compared with acid igneous rocks, basic and intermediate igneous rocks such as basalts and andesites are generally known to contain higher concentrations of elements such as Cu, Zn, Cr, Co and Ni, which occur mainly in the easily weathered constituents (He *et al*., 2005; Kabata-Pendias, 2001). However, Campbell (2009), who studied the effect of several types of rockdust from different origins, observed a decrease in the concentration of some elements in the soils by adding rockdust and described this effect as "unexpected". The present thesis was able to explain that "unexpected" decrease as a dilution effect (Paper I). Determination of the concentration of elements in the soil before and after treatment revealed that the significant differences observed in the concentrations of many elements between rockdust- and quartz sand-treated soils were due to dilution and not to enrichment, in which quartz sand was more effective. Data on the concentrations of EDTA-extractable elements in the soils confirmed the "dilution" rather than the "enrichment" effect of rockdust for trace elements, *e.g*. Cd and Pb concentrations were significantly higher in the low-rate rockdust application than in the high-rate rockdust and all quartz sand treatments. Thus any concerns about increasing potential toxic elements in soils by applying this rockdust are negligible. When rockdust was tested on nutrient-poor soils (Hollsby and Rådde) with lower soil pH (Paper II), Mg_{EDTA} was found to be enhanced in both soils compared with the unamended control, although the concentration was still low. This can be attributed to the much lower inherent/original Mg content of the soils, as well as the pH difference between these soils (pH ~5.5) compared with the soils used in Paper

I (pH ~6.5). The amount of Mg added by rockdust per pot (Paper II) was higher than with the other amendments and may be related to its content in, and release from, pyroxene and/or trioctahedral clay minerals, which were relatively abundant in the material. Hence, the initial concentration of nutrients in the rockdust, the type of minerals in which they are contained and the properties of the soil are all important factors determining the effectiveness of rockdust as a soil nutrient amendment.

5.1.3 On harvested biomass, botanical composition and nutrient quality of the crops

Rockdust and rock powders are reported to be slow-release sources of nutrients that raise soil fertility in the medium to long term (Winiwarter & Blum, 2008). In this thesis, the effect of rockdust on crop yield and element concentration was examined one and three years after rockdust application, to test both the short and longer term effects. The results showed that when soils had similar or higher concentrations of elements than rockdust, the yield did not improve either one year (wheat) or three years (mixed stand of ryegrass-red clover) after application, as applying rockdust diluted the inherent elements in the soils rather than enriching the concentrations. Similarly, Jones *et al*. (2009) reported that the addition of fine-grained basaltic materials to compost and peat caused a significant 2-fold reduction in wheat biomass production, which can be attributed to the dilution effect of rockdust on compost and peat rich in nutrients in their study. However, when the rockdust was tested on nutrient-poor soils, the harvested forage biomass was significantly higher than in the unamended control. A significant increase in wheat biomass grown on poor soils treated with biotite-containing granite has also been observed by Hinsinger *et al*. (1996).

Rockdust did not increase the concentrations of most nutrients, but enhanced plant tissue concentrations of Na in all soils and Mg in the less fertile soils compared with the control. This can be explained by the abundance of Na in rockdust, probably due to the presence of plagioclase feldspars, and by the fact that Na is known to be taken up quite readily if available in the soil (Whitehead, 2000). In the case of Mg, the capacity of the rockdust to supply this nutrient was significant due to the lower Mg concentration in the soils (Hollsby and Rådde). Hence, the importance of the original nutrient status of the soils for the scope of nutrient supply from amendments was once again highlighted and from my studies it is only nutrient poor soils that are likely to benefit from rockdusts.

5.2 Effect of biogas digestate, pot ale and wood ash as soil amendments

5.2.1 On soil microorganisms

Microbial responses to added C sources have previously been shown to be a sensitive way of detecting soil treatments such as organic amendments and restoration practices (Chapman *et al*., 2007). However, there was no clear effect on the physiological profiles of the soil microbial community caused by the amendments applied here. A clear change was observed due to the fully fertilised treatment, showing that microbial communities in Hollsby and Rådde responded to the higher element concentrations in their environment. The *Rhizobium/Agrobacterium* community, regardless of soil differences, was also affected by the availability of nutrients provided through the fully fertilised treatment. The type of crop planted is known to affect the microbial communities of the soil due to the different root exudates produced and excreted into the soil environment (Hartman *et al*., 2009). Therefore, the composition and total density of the plant community could be expected to affect the composition of the microbial community. Application of amendments led to a significant shift in botanical composition of the mixed stands, as N-free amendments (*i.e*. rockdust and wood ash) produced more clover relative to grass than the N-containing treatments (*i.e*. pot ale and fully fertilised treatment). The proportion of clover produced on soils treated with biogas digestate was similar to that produced by the N-free amendments, despite the fact that biogas digestate also contained N. Comparing the botanical composition produced by the two organic amendments suggests that more of the pot ale N than the digestate N was available to crops approximately one year after application. However, in spite of the differences in botanical composition and N availability, there were no significant differences in microbial physiological profiles (except in the fully fertilised treatment). Similarly, Marshall *et al*. (2011) reported that in the northern Canadian grasslands, plant functional group identity had little influence on the soil microbial community as measured by SIR and phospholipid fatty acids (PLFA). Direct changes in soil nutrients brought about by full fertilisation, in contrast, resulted in a decrease in soil microbial metabolic diversity as measured by SIR. This suggests that short-term influences of plant identity on soil, such as potential differences in the composition of plant root exudates, do not have a large impact on the soil microbial community, but that nutrient availability may do so. In an incubation study, Söderström (2012) found that addition of single macronutrients and micronutrients to the same soils (Rådde and Hollsby) did change the physiological profiles. Thirteen weeks after

addition, Mg and K (Hollsby) and N (both soils) had produced a physiological profile that differed from that of soil treated with any of the other nutrients. This suggests that at least some of the species in these microbial communities are indeed nutrient-limited, supporting the original hypothesis concerning nutrient limitation of microbes and plants. This further suggests that mineral nutrients do have a direct effect on microbial communities, as opposed to nutrients having an effect on plants that in turn influences the microbial community composition. However, an indirect effect via the plants cannot be ruled out, in particular for the *Rhizobium/Agrobacterium* community. These organisms may be expected to respond more closely to changes in legume density and dependency on N_2 fixation (Reverchon *et al*., 2012).

5.2.2 On soil properties and element status

Wood ash had a strong liming effect (Table 2) and indeed significantly increased the pH of the soils after application. It is presumed that portlandite and periclase, which were found in wood ash by XRD analysis (Paper II), contributed to its high liming effect. The presence of portlandite and periclase in wood ash has been attributed to the transformation of carbonates during combustion (Holmberg & Claesson, 2001). Wood ash had high total concentrations of most elements, *e.g.* Ca_{tot}, K_{tot} and Mg_{tot} (in agreement with Demeyer *et al*., 2001), which often exceeded those of the other amendments (Table 2). It was also reflected in the EDTA-extractable concentration of these elements in the soils. However, the actual amount of Ca and K added to each pot by wood ash according to the application rate was less than that added by rockdust (Paper II). The larger fertilising effect can be due to the different forms of these elements added through rockdust and wood ash and/or to the increase in soil pH by wood ash. Wood ash K is very soluble in water (Demeyer *et al*., 2001) and the solubility of Ca, K and Mg improves slightly when the pH increases from 5 to 6. The rise in pH after applying wood ash to the soils may have reduced the availability of micronutrients as it is known that *e.g.* Mn^{2+} in soil solution decreases 100-fold for each unit increase in pH (Havlin *et al*., 1999). An increase in Cd with wood ash has been investigated as a concern in many studies (Aronsson and Eklund, 2004) but the wood ash in my study did not increase available Cd.

5.2.3 On harvested biomass, botanical composition and nutrient quality of the crops

Wood ash, biogas digestate and pot ale all increased the biomass yield by on average 1.3-fold compared with the unamended control. While this is a benefit, it was still less than the 2.5-fold increase obtained with the fully fertilised

treatment. The effect of wood ash on the concentrations of elements in plant tissues was potentially either directly through nutrient addition (K, Mg, Mo) and/or indirectly through affecting soil pH. Concentrations of Ca, Mg, K and P have often been found to increase in response to ash application (Nkana *et al*., 1998; Krejsl & Scanlon, 1996; Etiegni *et al*., 1991). No increase in P was found in wood ash-treated plant tissue samples, probably due to the relatively high original P concentration of the soils. As wood ash increased the pH of the soils, it probably decreased the availability of micronutrients in the soils except for Mo, which is known to be more available at higher pH. A pH increase may reduce micronutrienst but will also reduce the availability of potentially toxic elements such as Cd and Pb, especially if they are already available in fairly high concentrations in the soils. Consequently a balance may have to be struck in considering the nutrient/contaminant status of each in the soil. The N fertilising effect of biogas digestate was small one year after application although the growth of clover was more enhanced than the grass. However, plant (grass and clover) Ni concentration was higher than in the control, suggesting that the bioavailability of Ni in the digestate was high. Valeur (2011) found Ni to be the most labile of the trace elements examined in biogas digestates and suggested that plant availability of Ni could thus be considered high. Ni is an essential trace element for plants (Chen *et al.*, 2009) and so this amendment would be particularly suitable for Ni deficient soils. In contrast soils with already high lithogenic Ni e.g. serpentine soils (Proctor 1971) would be a less suitable for receiving biogas digestate. Complexation reactions are known to occur during digestion in bioreactors and may strongly increase the bioavailability of metals (Callander & Barford, 1983). In contrast to the biogas digestate, the lack of any stimulating effect on clover growth by the pot ale suggests that it is less suitable as a fertiliser on leys containing clover as an important component. The high Cu concentration of the pot ale, the subsequent high Cu application rate and the high concentrations of Cu_{EDTA} were not reflected in the plant Cu concentrations. These even fell within the suggested concentration range of Cu deficiency for the ryegrass, indicating that the Cu provided by the pot ale was not readily plant-available.

5.3 Environmental drivers of microbial community composition at a landscape scale

Soils from across a landscape will have high diversity in properties including minerals and nutrient contents and I had hypothesized that as long as other major drivers e.g. pH were within a narrow range that the variation in community composition would be explained by mineralogy and elemental

content. In fact a high percentage of the variation observed in the physiological (79%) and genetic diversity (58%) of soil microorganisms could be explained by the selected range of environmental factors.

5.3.1 Minerals and element concentrations

Variance partitioning showed that the effect of minerals in explaining the variance in microbial communities is probably due to the contribution by minerals to *aqua regia*-extractable concentration of soil elements, as the effect of the 'Minerals' subset was non-significant after removing the variation caused by the element concentrations. *Aqua regia*-extractable element concentrations were the most important soil properties affecting the microbial communities. The Mn concentration of the soils was the strongest factor in explaining the variation in CLPP data (43.5%). This might be due to the role of Mn in redox reactions in the soils, which recent studies suggest have been underestimated (Husson, 2013). Facultative anaerobes can utilise manganese compounds (Mn^{2+}) as electron acceptors during their metabolic activity (Reddy *et al*., 1986). Overall, trace elements in the soil (including some essential micronutrients) do seem to have an influence on the microbial community composition, as measured by CLPP and on *Rhizobium/Agrobacterium* genotypes.

5.3.2 Other environmental variables

Many studies have shown that soil pH is the main variable in explaining microbial community composition (*e.g.* Fierer & Jackson, 2006). Yao *et al*. (in press) also reported pH to be the major driver of ammonia oxidiser communities, in a study using samples with a much wider range of soil pH (3.1- 7.4). In this thesis, soils with a narrower pH range (4.5- 6.6) were selected in order to factor out pH as a major driver and thus isolate the role of other factors more easily. This was largely accomplished as there was no dominant effect of pH observed either on the CLPP or T-RFLP data. I found that rainfall is an important factor for *Rhizobium/Agrobacterium* genotypes. Rainfall was also shown to be among the important drivers of ammonia-oxidising archaea and bacteria in the soils sampled across Scotland (Yao *et al.*, in press). Clearly, rainfall patterns could have an important effect on the water relations and aeration of soils, and thus on the diversity of these taxonomic groups. However, the influence of rainfall pattern might also be indirect, through its influence on vegetation and in particular the host plants associated with *Rhizobium/Agrobacterium* (Reverchon *et al*., 2012). *Rhizobium/Agrobacterium* genotypes were found to be discriminated even between fairly similar land uses, namely permanent and rotational pastures, suggesting that despite broadly

similar pasture species, the frequency of cultivation and other management differences may have caused changes in microbial community composition. Palmer & Young (2000) demonstrated that arable fields subjected to repeated cultivation had higher rhizobial genetic diversity (focusing on pea-nodulating *R. leguminosarum* biovar viciae) as relatively undisturbed grasslands. The results thus indicate that land use can have an effect on microbial communities, corroborating findings by Mitchell *et al*. (2010) and Yao *et al*. (2000). However, different functional and taxonomic groups vary in their responses. For example, Yao *et al*. (in press) reported that ammonia-oxidising archaea and ammonia-oxidising bacteria communities were not significantly different in the arable and pasture soils included in this thesis. Rhizobia were the main interest in this thesis due to their importance for N_2 fixation in grasslands and so variation in clover and the association with Rhizobium could explain why I found differences in land use when Yao et al (in press) did not.

6 Conclusions

The main aim of this thesis was to investigate how soil microbial communities are influenced by the macronutrient and micronutrient status of the soil, as determined by inherent soil properties and nutrient addition. The effect of nutrients added via by-products in order to increase crop biomass production and nutritional quality was also investigated.

The results showed that the rockdust product used did not offer any added benefit to wheat or grass-clover grown in relatively fertile soils three years after being applied. Rockdust did not enhance the grain yield of wheat or the biomass yield of grass-clover mixed stands on two agricultural soils and one garden peat measured at one or three years after application. However, when it was applied to two nutrient-poor soils, the biomass yield of grass-clover stands was enhanced one year after application. This shows that in addition to the original content of macronutrients and micronutrients in the by-products, the baseline status of the soil in terms of nutrient elements is also a determining factor for the success of soil amendments in enhancing crop yield and quality.

The results also showed that each of the different amendments tested offered some benefit in supplying different macronutrients and micronutrients when applied to low nutrient soils (Paper II and III). In short-term experiments there appeared to be little effect on the composition and physiological responses of soil microorganisms, as the microbial community of the different soil types did not differ considerably after application of amendments. It can thus be concluded that at least in the short term, the amendments would not have adverse effects on soil microbes in agricultural fields, given that the type of amendments tested with the rates applied in these experiments did not increase the concentration of potentially toxic elements (Cd, Pb) or other trace elements. Nevertheless the possible long-term effects of repeated application of these by-products to soils should be tested further.

There were benefits from the by-products (biogas digestate, pot ale, wood ash) on the two nutrient-poor soils studied, e.g. in terms of increasing the biomass yield, but their benefits in terms of soil pH and EDTA-extractable nutrients were not substantial. Wood ash can act as a neutralising agent when applied to acid soils and can also increase Ca_{EDTA}, K_{EDTA} and Mg_{EDTA} in the soil. However, it can also decrease the availability of micronutrients due to the increase in pH, which would be of concern for micronutrient-deficient soils. None of the amendments was successful in increasing the available concentrations of micronutrients except for pot ale, which increased Cu_{EDTA}. On the other hand, with the rates applied, the amendments did not increase the EDTA-extractable concentration or availability of potential toxic elements in the soils. The by-products all increased overall crop biomass production, but had distinctly different effects on the individual plant species and hence the botanical composition and the macronutrient and micronutrient composition of the mixture. There would appear to be some potential benefits from matching different by-products to different soils in order to enhance agricultural productivity through improved crop quality. However, a full systems analysis approach is needed to secure safe and long-term sustainable use of by-products as fertilisers.

Several environmental variables appeared to explain significant proportions of the variation in physiological and genetic profiles of microbial communities in a landscape scale. Mn_{AQR} and rainfall were the main factors explaining the variation in the physiological profiles and *Rhizobium/Agrobacterium* genotypes, respectively. In general, micronutrients and non-nutrient elements contributed more to the variation in microbial data than other environmental variables. There are a number of factors which require further exploration to confirm that the micronutrients found here to be significant explanatory variables are underpinned by an understandable mechanism. For example we only measured *aqua regia*-extractable concentrations, but different extractants could give better indications of the availability of micronutrients. This would be an obvious next step in our investigations. *Rhizobium/Agrobacterium* genotypes proved to be discriminative even between fairly similar land uses, namely permanent and rotational pastures, suggesting that the frequency of cultivation and other management differences caused changes in community composition.

Overall, this thesis showed that mineral and organic amendments can be used to modify the nutrient status of soils for the benefit of crops if specific knowledge of the amendment and the soil is taken into consideration. It also showed that plants and microorganisms are influenced by macronutrient and micronutrient concentrations and this too needs to be considered more fully in

studies of soil-plant-microbial systems and their responses if we are to fully meet the challenge of food security.

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