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ORIGINAL ARTICLE

Assessing biogas digestate, pot ale, wood ash and rockdust as soil amendments: effects on soil chemistry and microbial community composition

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Applying by-products as soil amendments to agricultural systems is growing in popularity. We aimed to assess the efficacy of some contemporary by-products to provide nutrients to crops as well as the potential harm of adding toxic elements to the environment. Four different by-products widely available in Northern Europe were tested for their effects on two nutrient-poor agricultural soils in terms of increasing available macro- and micronutrients as well as toxic elements. Assessing soil microbial community as a sensitive tool for evaluating soil quality was conducted with the focus on microbial activity, carbon metabolism and on *Rhizobium/Agrobacterium*. Wood ash increased pH and Ca_{EDTA}, K_{EDTA} and Mg_{EDTA} in the soils. The only increase in EDTA-extractable micronutrients in the soils was observed by applying pot ale, increasing Cu. None of the amendments increased the availability of Pb and Cd in the soils. Soils amended with the by-products thus remained similar to the unamended control but were quite different from fully mineral fertilised soils. There were no detectable adverse effects on the physiological and genetic profiles of microbial communities. The by-products were moderately beneficial and did not change the soil microbial community as much as the fully fertilised treatment with mineral fertilisers. Changes in the microbial community profiles were probably due to direct effects on microbes limited by K, Ca and N as opposed to indirect effects on plant growth. This is potentially significant in understanding how to improve impoverished and marginal soils as microbial activity affects many other ecosystem functions.

Keywords: macro elements; MicroResp; nutrient deficiency; substrate-induced respiration; trace elements; T-RFLP

Introduction

Ever-growing environmental awareness together with economic pressures has led to an interest in finding ways of recovering and re-using nutrient resources in agriculture in more efficient ways. This includes options for utilising by-products from industrial and domestic activities and energy production as nutrient and organic matter sources for land application. In addition, the demand for organic agricultural products using naturally available organic and inorganic nutrients – often only slowly available – results in a need to test the efficacy and potential of such by-products as soil amendments and plant nutrient sources. However, at the same time as enhancing the nutrient status of the soils, these by-products can potentially add toxic elements such as Cd, Hg (Park et al. 2012), As, Ni, Pb (Yunusa et al. 2012) or alter soil properties e.g. increasing soil pH and EC (Park et al. 2012) in ways that may harm the soil and related environments if used repeatedly over the long term.

There have been several studies concerning the application of waste products such as biogas digestate (Haraldsen et al. 2011; Losak et al. 2014;

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Svensson et al. 2004), pot ale (Bucknall et al. 1979; Douglas et al. 2003), rockdust (Coroneos et al. 1996; Campbell 2009; Ramezanian et al. 2013) and wood ash (Pitman 2006; Agbede & Adekiya 2012) for fertilising crops, vegetables and trees. However, most studies have examined the effects on fertile soils and few studies have considered how to match specific nutrients to low-fertility soils, especially soils low in micronutrients. While there are several potentially useful by-products, their efficacy and associated risks all need to be evaluated more carefully. This includes understanding not only the effects on plant growth and the plants' nutrient content, but also the wider ecological effects, e.g. on the soil biota which are responsible for many of soil's functions (Arthurson 2009; Abubaker et al. 2015). As they respond rapidly to the stressors, soil microorganisms are among the most studied indicators for evaluating soil quality (Schloter et al. 2003; Ritz et al. 2009).

In this study, the by-products biogas digestate, pot ale, rockdust and wood ash were selected as they are produced in many parts of the world, including northern Europe. In addition to effects on soil chemistry, the effects on soil biota were also tested. Two methods for assessing the soil microbial community were used, namely community-level physiological profiles (CLPP) by MicroResp™ and genetic profiles by terminal restriction fragment length polymorphism (T-RFLP). The former measures the carbon substrate use as substrate-induced respiration (SIR) of the soils. The latter is a DNA-based molecular fingerprinting method and was here targeted at *Rhizobium*/*Agrobacterium* genotypes. In many previous studies, the effects of by-products have been tested in agricultural soils that conventionally would be regarded as better-quality soils in terms of plant growth (e.g. Baerug 1991b; Erich 1991; Huang et al. 1992; Ramezanian et al. 2013).

In this study, the aim was to test the benefits and risks of applying the by-products biogas digestate, pot ale, rock dust and wood ash as amendments to nutrient-poor soils on the basis that such soils are more likely to show a benefit as regards fertiliser effects. The specific objectives were: (1) to evaluate the potential effect of applying different by-products as soil amendments in terms of improving soil pH and available macro- and micronutrients; (2) to quantify the possible addition of potential toxic elements and assess their solubility (EDTA extractability); and (3) to assess their effect on soil quality in terms of microbial community function and detect possible alterations in important microbial genotypes in soils. The effects on *Rhizobium*/*Agrobacterium* genotypes were specifically tested as these are not only important species in forage pastures for symbiotic N fixation in clover, but also because they are known to respond to micronutrient deficiencies (Vincent 1982) and to be sensitive to over-application of heavy metals (Chaudri et al. 1993). Effects of these by-products on plant growth, chemical composition and proportions of grass and clover in the mixed study crop were also evaluated and are reported in Dahlin et al. (2015).

Materials and methods

Selected soils and amendments

Two nutrient-poor top soils (0–0.20 m) from Swedish agricultural lands were chosen for this experiment: Rådde (57°36′N, 13°15′E) which is a till with sandy loam texture developed from gneissic and granitic parent material, and Hollsby (59°48′N, 13°31′E) which is a post-glacial silt loam originating from mainly granitic and sandstone bedrock (The Geological Survey of Sweden). Soil organic carbon was 2.2% and 3.5% for Rådde and Hollsby, respectively, whereas pH_{H2O} (5.4 vs. 5.5), pH_{CaCl2} (4.8 vs. 5.1) and the C:N ratio (12 vs. 13) for Hollsby and Rådde were fairly similar (Table 1). The geochemistry of the soils is shown in Table 1. Prior to the experiment, both soils were sieved through an 8 × 18 mm aluminium mesh and thoroughly mixed.

A range of organic and inorganic soil amendments (Table 1) were compared to an unamended control and a fully fertilised treatment in order to test their efficiency at improving the soil status of potentially bioavailable nutrients and the potential effect on soil microbial communities. The amendments used in this experiment were biogas digestate obtained from a biogas plant in Sweden fed source-separated household wastes and grass silage, a composite pot ale from several whisky distilleries in Scotland, commercially available volcanic rockdust from the SEER Centre in Perthshire, Scotland and bottom ash produced following the combustion of mixed deciduous wood on a farm in central Sweden (Table 2). Since the main limiting factor for plant growth on wood ash and rockdust-amended soils was assumed to be nitrogen (N), additional treatments of wood ash/rockdust + N at 50 + 35 + 20 kg ha^{-1} (split applications) were included. A fully fertilised treatment (Table 2; split application three times and two times per season in years 1 and 2, respectively) and an unamended control were included for reference purposes. The amendments' application rates were calculated according to the total concentrations of the elements in the amendments and the maximum allowable seven-year application rates of nutrient and non-nutrient elements for trace elements (Cd, Cr, Cu, Hg, Ni, Pb, Zn) as stated by the Swedish

Table 1. Properties of the soils and organic/inorganic amendments used in the experiment.

		Soils		Amendments			
Parameters	Unit	Hollsby	Rådde	Biogas digestate	Pot ale	Rockdust	Wood ash
Clay	%	4	8	–	–	–	–
Silt	%	69	31	–	–	–	–
Sand	%	27	61	–	–	–	–
pH-H_2O	–	5.4	5.5	–	–	–	–
pH-$CaCl_2$	–	4.8	5.1	–	–	–	–
Liming effect	%CaO	–	–	6.2	−4	1.9	5100
C_{tot}	%	2.2	3.5	42	47	0.005	1.1
N_{tot}	%	0.19	0.28	**2.4**[a]	**5.8**[a]	0.097	0.01
Ca_{tot}	g kg^{-1}	1.0	11	48	1.7	13	324
Fe_{tot}	g kg^{-1}	16	18	3.7	0.05	31	10
K_{tot}	g kg^{-1}	25	21	12	32	2.6	69
Mg_{tot}	g kg^{-1}	3.0	3.5	6.2	6.0	17	40
P_{tot}	g kg^{-1}	0.78	1.2	8.3	15	1.2	21
P_{HCl}	g kg^{-1}	0.61	0.84	–	–	–	–
S_{tot}	g kg^{-1}	0.33	0.48	3.1	4.2	0.094	0.82
Cd_{tot}	mg kg^{-1}	0.12	0.13	0.10	0.02	0.039	0.27
Co_{tot}	mg kg^{-1}	2.6	3.8	0.89	0.07	12	21
Cr_{tot}	mg kg^{-1}	15	23	13	0.13	12	74
Cu_{tot}	mg kg^{-1}	6.9	6.5	29	177	7.3	118
Mn_{tot}	mg kg^{-1}	531	431	215	16	375	7810
Mo_{tot}	mg kg^{-1}	0.40	0.85	1.9	0.45	0.20	<6
Ni_{tot}	mg kg^{-1}	4.4	7.1	5.6	0.41	9.7	**121**[a]
Pb_{tot}	mg kg^{-1}	18	19	9.1	2.9	2.5	2.4
Zn_{tot}	mg kg^{-1}	46	30	76	21	46	182

[a]Values in bold show the limiting element in each amendment.

Environmental Protection Agency (SEPA 1994), and following the fertiliser recommendations of the Swedish Board of Agriculture (2011) for P and N. However, rockdust was applied according to the highest rate of application recommended by the suppliers, i.e. 5 kg m^{-2}. For the latter, this rate actually meant exceeding the maximum allowable Ni and Cr addition rates (SEPA 1994), but as the availability of elements was expected to be low, the supplier's recommendations were observed. The fully fertilised

Table 2. Elements added to soils (in pots) by amendments and fully fertilised treatment.

		Fully fertilised treatment						
Elements	Unit kg^{-1} soil	Year 0	Year 1	Year 2	Biogas digestate	Pot ale	Rockdust	Wood ash
C	g				1.5	1.07	0.0	0.013
N	g	0.087	0.082	0.082	0.082	0.13	0.0	6×10^{-6}
Ca	g	6×10^{-5}	6×10^{-5}	6×10^{-5}	0.17	0.004	0.54	0.387
Fe	g	0.003	–	–	0.46	0.031	12.9	0.12
K	g	0.18	0.18	0.18	0.041	0.071	0.11	0.083
Mg	g	0.081	0.081	0.081	0.021	0.013	0.69	0.048
P	g	0.062	0.062	0.062	0.028	0.032	0.049	0.026
S	g	0.005	0.005	0.005	0.38	2.9	0.039	0.010
Cd	mg	–	–	–	4×10^{-4}	4×10^{-5}	0.007	3×10^{-4}
Co	mg	–	–	–	0.003	2×10^{-4}	0.485	0.025
Cr	mg	–	–	–	0.044	3×10^{-4}	3.0	0.014
Cu	mg	0.035	–	–	0.10	0.39	0.30	0.14
Mn	mg	0.81	–	–	0.74	0.04	12.3	9.3
Mo	mg	0.32	–	–	0.006	0.001	8×10^{-4}	0.004
Ni	mg	–	–	–	0.019	9×10^{-4}	0.40	0.14
Pb	mg	–	–	–	0.031	0.006	0.10	0.003
Zn	mg	1.3	–	–	0.26	0.046	1.88	0.22

treatment corresponded to the complete fertiliser treatment used in nutrient deficiency demonstrations on a range of crops at the Department of Soil and Environment, Swedish University of Agricultural Sciences (SLU) with a reduced N application rate in order to achieve a mixed grass-clover stand in the pots.

After the second harvest in 2010 (year 1), NPK was added to all pots, with the amendments at the same rates as the fully fertilised treatment, to enhance crop growth and increase chances of detecting the supply of other macro- and micronutrients from the amendments. However, N was not supplied to rockdust and wood ash treatments after the second harvest (since N treatment was part of the experiment's original design). All pots were again fertilised with NPK in 2011 (year 2): 100 kg ha^{-1} N and 30 kg ha^{-1} K split 45/35/20 and 18/14/8 during the season respectively and 35 kg ha^{-1} P as in the fully fertilised treatment. Nitrogen and K were added as solutions of NH_4NO_3 and KCl respectively, whereas P was added as dry $CaHPO_4{\cdot}2H_2O$ at the soil surface. The pots treated with wood ash only and rockdust only were subsequently discontinued.

Establishment, harvesting and sampling of pot experiment

At establishment, 7 L plastic pots (0.22 m inner diameter, 0.25 m depth) were filled with fresh soil corresponding to 6 kg DW and mixed with the respective amendments in four replications in late July 2009 (the establishment year). Pots were sown with a mixed stand of perennial ryegrass (*Lolium perenne* L., cv. Helmer) and red clover (*Trifolium pratense* L., cv. Nancy) with 20 seeds of each per pot which were thinned to 10 plants of each species two weeks after emergence. The pots were kept under semi-natural conditions in an open netted yard in a completely randomized design. Natural precipitation was complemented by irrigation with deionised water where necessary. Plants were not cut during the establishment year (year 0), and pots were placed in a cold room (−1°C to +1°C) for over-wintering in late November and returned to the outdoor yard in April. The swards were cut three and two times in years 1 and 2, respectively. Soils were sampled at the end of the growing season of both years 1 and 2 by taking 7–10 random cores per pot. Collected soil samples were homogenised and divided into three plastic bags which were then either (1) air dried and sieved to <2 mm using a plastic sieve for chemical analyses, (2) stored fresh at +4°C for MicroResp™ or (3) frozen at −80°C for DNA extraction to perform T-RFLP.

Chemical and mineralogical analyses of soils and amendments

Total elemental analyses of the original soils and the amendments were done at the ALS Scandinavia AB laboratory in Luleå, Sweden. Soil samples, wood ash and rockdust were digested in 6 ml HNO_3 + 2 ml HCl + 2 ml HF (concentrated) in closed Teflon containers in a microwave digestion system, after which cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), lead (Pb), sulphur (S) and zinc (Zn) were measured using ICP-SFMS, fused with lithium metaborate, then dissolved in HNO_3 and calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na) and phosphorus (P) measured using ICP-AES. Biogas digestate and pot ale were digested in 5 ml HNO_3 and 20 µl HF (concentrated) in open vessels in a microwave oven and all elements measured using ICP-SFMS. Total concentration of elements will be referred to as Ca_{tot}, etc.

The following analyses were performed in the Department of Soil and Environment at SLU. In all cases, an in-house reference soil was included in all the batches for quality assurance. Electrical conductivity (EC) and the pH in deionised water (pH_{H20}) and in 0.01 M $CaCl_2$ (pH_{CaCl2}) were measured in a sequence as follows: 15 g of soil was mixed with 30 ml deionised water and shaken for 1 hour and left to stand overnight, after which time the EC was measured. The suspension was stirred for 30 seconds and then the pH_{H2O} (1:2) was measured. 0.3 ml of 1 M $CaCl_2$ was added to the suspension and stirred intermittently for 30 minutes and the pH_{CaCl2} was measured (Sumner 1994). The total N and C concentrations in the soil and amendment samples were analysed by high-temperature induction furnace combustion using LECO CN2000 (LECO Corporation, St. Joseph, MI, USA). To compare the soil status with Swedish monitoring data (Eriksson et al. 2010; Swedish Monitoring Program 2013), HCl-extractable P (P_{HCl}) was also determined in the original soils according to KLS (1965). As a proxy for plant-available elements, EDTA-extractable elements (Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, S, Zn) of the soil samples collected in the autumn of year 1 were measured using the method described by Streck and Richter (1997) with modifications so that Na-EDTA was used and the extracts analysed using ICP-MS ELAN 6100 DRC (Perkin Elmer SCIEX, Waltham, MA, USA). The results from these extractions are referred to as Ca_{EDTA}, etc.

Mineralogical analyses were carried out at The James Hutton Institute in Aberdeen, Scotland. For quantitative and qualitative mineralogical analysis,

3 g each of air-dried soil and amendment samples were ground in an agate McCrone mill in ethanol for 12 minutes. Random powders were prepared by spray drying the resulting slurries following the procedure described by Hillier (1999). X-ray Diffraction (XRD) patterns for quantitative analyses of the two soils were measured on spray-dried samples run on a Siemens D5000 (Siemens, Germany) using Co K-alpha radiation selected with a graphite monochromator, and analyses conducted using a full-pattern fitting method (Omotoso et al. 2006). The XRD patterns for qualitative analyses of spray-dried amendment samples were recorded on a Panalytical Xpert Pro diffractometer (Panalytical, the Netherlands) using Ni-filtered Cu K-alpha radiation.

Soil microbiological analyses

The physiological response of the soil microbial communities was measured as the flush of carbon dioxide after the addition of 15 different carbon sources (substrates), usually called SIR, and was determined using the MicroResp™ method (Campbell et al. 2003) on soil samples taken at the end of the growing seasons in year 1 and year 2, similarly to that described by Ramezanian et al. (2013).

T-RFLP is a molecular technique that digests soil microbial community DNA into fragments that can be identified on a DNA sequencer by their fragment size. The range and relative abundance of fragment sizes reflects differences in microbial genotypes in the community. DNA was extracted from 0.5 g of soil samples taken in year 1 using the FastDNA SPIN kit (MP Biomedicals LLC.) as described by Berthelet et al. (1996). In-house reference soil samples were also included for quality control purposes. T-RFLP was mainly performed in accordance with Singh et al. (2006). Briefly, 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, UK) 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 HhaI (Promega, UK). 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 a 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 as well as a positive control (100% *Rhizobium*) were run on the Applied Biosystems 3130xl Genetic Analyser. T-RFLP profiles from each sample were obtained using GeneMapper v4.0 software (Life Technologies Corporation). In total, 50 fragments were obtained across all samples. Fragments at or close to the limit of detection and/or only found in less than 10% of samples were omitted from the analysis, leaving 29 fragments used in the multivariate analysis.

Statistical analyses and data evaluation

Treatment differences in the soil chemical data were assessed by analysis of variance (ANOVA) with Tukey pair-wise comparisons using JMP 9.0.0 (SAS Institute Inc., Cary, NC, USA) and $p < 0.05$ as the limit for statistical significance. Some of the observations were log transformed before the analysis to obtain normal distribution of the residuals. Statistical analysis of CLPP data from all added C sources was based on Euclidean distances of SIR data which were analysed by canonical variate analysis using CAP software (Anderson & Willis 2003). Each variable (C sources) was standardised by dividing it by its standard deviation. Fragment data obtained from T-RFLP analyses were converted into a binary data table (presence or absence of individual peaks) using MS Excel and analysed by canonical variate analyses based on Bray–Curtis dissimilarities using CAP software (Anderson & Willis 2003) to identify patterns of microbial communities in treatments.

Results

Properties of soils and amendments

The dominant constituent minerals of the two soils selected for the experiment were quartz, K-feldspar and plagioclase with a cumulative weight percentage of 89% and 86% of fine earth (<2 mm) by weight for Hollsby and Rådde respectively (Table 3). Total phyllosilicates accounted for around 8% of both soils, just less than half being trioctahedral. Both soils also contained some amphibole (1.8 and 4.3%), the concentration being highest in Rådde. Qualitative XRD studies of the amendments (XRD patterns are given in the Supplemental data, Figures S.1–S.4) detected a range of minerals in the rockdust, wood ash and biogas digestate. The rockdust mostly

Table 3. Mineralogical composition of soils in <2 mm fraction (%).

	Quartz	K-feldspar	Plagioclase	Amphibole	Di-phyllo.	Tri-phyllo.	Iron oxides	Total
Hollsby	52.9	16.8	19.0	1.8	4.5	3.3	1.5	100
Rådde	51.7	15.3	19.2	4.3	4.4	4.1	1.1	100

Note: Di-phyllo, dioctahedral phyllosilicates; Tri-phyllo, trioctahedral phyllosilicates.

contained plagioclase and some clay minerals, K-feldspar, quartz and pyroxene (Ramezanian et al. 2013). Quartz and feldspars were also found in the wood ash, along with portlandite ($Ca(OH)_2$), periclase (MgO) and di-calcium silicate ($Ca_2(SiO_4)$). The biogas digestate contained quartz and feldspars, along with some calcite ($CaCO_3$) and struvite ($MgNH_4PO_4(H_2O)_6$) as well as substantial amorphous organic matter. No inorganic mineral phases were detected in the pot ale by XRD analysis which was determined to be composed entirely of a mixture of (unidentified) crystalline and amorphous organic compounds.

The amendments selected had specific characteristics of interest for the purposes of this study. Wood ash had the highest liming effect among the amendments (Table 1). Furthermore, with few exceptions wood ash had higher total concentrations of most nutrients and trace elements than other amendments (Table 1). However, for most elements the amount added to the individual pots regarding the application rate for each of the amendments is highest in rockdust (Table 2). Pot ale had high concentrations of N and Cu, and biogas digestate had a fairly balanced nutrient content with N in available forms (Table 1).

Soil chemistry

pH_{H2O} and pH_{CaCl2} after growing season 1 were significantly different in the two soils, with Hollsby having a higher value than Rådde (Table 4). Among the treatments, wood ash and wood ash + N resulted in significantly higher pH_{H2O} and pH_{CaCl2} than the other treatments (Table 4).

EDTA-extractable Ca, Cd, Cr, Cu, Fe, K, Mg, Ni and P were significantly higher in the Hollsby soil than the Rådde soil (Tables 5 and 6). Wood ash increased Ca_{EDTA}, K_{EDTA} and Mg_{EDTA}, pot ale increased K_{EDTA} and Mg_{EDTA}, and rockdust only increased Mg_{EDTA} significantly compared to the unamended control in both soils (Table 5). None of the amendments increased P_{EDTA} and S_{EDTA} in soil samples in comparison with the unamended control (Table 5). The only soil amendment tested which increased EDTA-extractable concentrations of a micronutrient was pot ale which increased Cu_{EDTA} by factors of 1.4 and 1.3 respectively compared to the unamended control in Hollsby and Rådde (Table 6). None of the amendments increased the soils' Cd_{EDTA}, Pb_{EDTA} and Ni_{EDTA} (Table 6).

Soil biological analyses

Most of the C sources resulted in detectable SIR in all treatments, with alpha-ketoglutaric acid producing the highest SIR in soils in all treatments and in both years, i.e. 14 and 26 months after the amendment application (Figures 1 and 2). Significant differences in SIR for the different C sources were observed between the soils of Hollsby and Rådde, and this was evident in both years. The SIR of each C source was mostly highest in pot ale followed by the fully fertilised and wood ash + N treatment, while rockdust treated soils consistently had the lowest SIR for any of the C sources (Figure 1). However, in soil samples collected 26 months after application (year 2), the fully fertilised treatment produced the highest SIR from most C sources, followed by pot ale (Figure 2). The multivariate analysis of the CLPPs obtained by SIR data showed that the greatest difference was between the two different soils and was primarily on CV 1, which explained 65% of the variation in soil samples taken in years 1 and 2 (Figures 3 and 4). The physiological

Table 4. pH of soils after harvesting year 1 (n = 4).

	pH-H_2O	pH-$CaCl_2$
Soils	⋆	⋆
Hollsby	5.6^{A}	5.1^{A}
Rådde	5.4^{B}	4.8^{B}
Treatments	⋆	⋆
Unamended	5.4^{b}	4.8^{d}
Fully fertilised	5.4^{b}	5.0^{bc}
Biogas digestate	5.5^{b}	4.9^{cd}
Pot ale	5.4^{b}	4.8^{cd}
Rockdust	5.5^{b}	4.9^{cd}
Rockdust + N	5.5^{b}	4.9^{cd}
Wood ash	5.8^{a}	5.2^{ab}
Wood ash + N	5.8^{a}	5.3^{a}
Soil × treatments	ns	ns

⋆$p < 0.001$.
Note: ns, not significant.
Uppercase letters refer to comparison of soils; lowercase letters refer to comparison of treatments as determined by ANOVA.

Table 5. EDTA-extractable macroelements in soils after harvest year 1 (n = 4).

	Macroelements (g kg^{-1} DW)				
	Ca	K	Mg	P	S
Soils	**	**	**	**	**
Treatments	**	**	**	**	**
Soil × treatments	**	**	**	**	*
Hollsby					
Unamended	0.99^{c}	0.020^{defg}	0.016^{gh}	0.026^{a}	0.012^{b}
Fully fertilised	0.77^{de}	0.030^{abcd}	0.040^{a}	0.027^{a}	0.029^{a}
Biogas digestate	1.08^{bc}	0.027^{bcdef}	0.016^{gh}	0.028^{a}	0.013^{b}
Pot ale	1.03^{c}	0.033^{abc}	0.021^{def}	0.029^{a}	0.012^{b}
Rockdust	1.00^{c}	0.020^{efg}	0.022^{de}	0.027^{a}	0.012^{b}
Rockdust + N	1.04^{c}	0.026^{bcdef}	0.026^{cd}	0.024^{a}	0.012^{b}
Wood ash	1.21^{ab}	0.034^{ab}	0.029^{bc}	0.027^{a}	0.012^{b}
Wood ash + N	1.26^{a}	0.042^{a}	0.033^{ab}	0.028^{a}	0.013^{b}
Rådde					
Unamended	0.55^{f}	0.015^{gh}	0.005^{k}	0.010^{bc}	0.010^{b}
Fully fertilised	0.82^{d}	0.029^{bcde}	0.040^{a}	0.029^{a}	0.034^{a}
Biogas digestate	0.65^{ef}	0.020^{fg}	0.007^{j}	0.014^{bc}	0.011^{b}
Pot ale	0.56^{f}	0.023^{cdef}	0.009^{i}	0.015^{b}	0.011^{b}
Rockdust	0.65^{ef}	0.013^{h}	0.014^{h}	0.011^{bc}	0.010^{b}
Rockdust + N	0.56^{f}	0.014^{h}	0.013^{h}	0.010^{c}	0.010^{b}
Wood ash	0.81^{de}	0.028^{bcdef}	0.018^{fg}	0.014^{bc}	0.009^{b}
Wood ash + N	0.82^{d}	0.034^{abc}	0.019^{efg}	0.013^{bc}	0.009^{b}

*p < 0.05; **p < 0.001.
Note: Different letters within the same column show a significant difference as determined by ANOVA.

profiles of the microbial communities differed less in response to applying the treatments. However, the fully fertilised treatment of both soils tended to cluster together separately from other treatments in their soil group (Figures 3 and 4). Furthermore, the physiological profiles of soil microbes were

Table 6. EDTA-extractable microelements in soils after harvest year 1 (n = 4).

	Microelements (mg kg^{-1} DW)									
	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
Soils	***	***	***	***	***	***	ns	***	***	***
Treatments	***	***	**	***	***	***	***	ns	ns	**
Soil × treatments	*	**	***	*	***	***	ns	ns	*	***
Hollsby										
Unamended	0.093^{a}	0.15^{cde}	0.059^{a}	1.4^{bcde}	0.17^{a}	26^{c}	0.020	0.89	2.0^{bcde}	3.6^{def}
Fully fertilised	0.073^{b}	0.21^{abc}	0.027^{b}	1.1^{g}	0.11^{ef}	64^{ab}	0.073	1.14	2.4^{ab}	5.4^{abc}
Biogas digestate	0.093^{a}	0.13^{de}	0.057^{a}	1.5^{bc}	0.16^{abc}	23^{c}	0.018	0.92	2.0^{bcde}	3.8^{bcdef}
Pot ale	0.094^{a}	0.13^{de}	0.059^{a}	2.0^{a}	0.16^{abc}	21^{c}	0.018	0.97	2.0^{bcde}	3.7^{cdef}
Rockdust	0.091^{a}	0.13^{de}	0.052^{a}	1.4^{bcdef}	0.17^{ab}	21^{c}	0.021	1.09	1.9^{cde}	3.4^{ef}
Rockdust + N	0.089^{a}	0.13^{de}	0.054^{a}	1.4^{bcde}	0.18^{a}	21^{c}	0.019	0.95	1.9^{de}	3.2^{f}
Wood ash	0.093^{a}	0.11^{e}	0.058^{a}	1.6^{b}	0.15^{abcd}	22^{c}	0.019	1.04	1.9^{e}	3.9^{bcdef}
Wood ash + N	0.094^{a}	0.11^{e}	0.055^{a}	1.6^{b}	0.15^{abcd}	22^{c}	0.019	0.96	1.8^{e}	3.6^{def}
Rådde										
Unamended	0.075^{b}	0.23^{ab}	0.031^{b}	1.1^{g}	0.12^{def}	72^{ab}	0.023	0.72	2.5^{a}	5.7^{ab}
Fully fertilised	0.069^{b}	0.21^{abc}	0.033^{b}	1.1^{g}	0.11^{ef}	62^{ab}	0.069	0.93	2.3^{abcd}	4.9^{abcde}
Biogas digestate	0.076^{b}	0.22^{abc}	0.031^{b}	1.2^{efg}	0.12^{def}	71^{ab}	0.023	0.83	2.5^{a}	5.4^{abc}
Pot ale	0.077^{b}	0.26^{a}	0.027^{b}	1.5^{bcd}	0.18^{efg}	86^{a}	0.019	0.80	2.6^{a}	5.6^{ab}
Rockdust	0.074^{b}	0.22^{abc}	0.027^{b}	1.2^{fg}	0.14^{cde}	66^{ab}	0.020	0.76	2.4^{ab}	6.8^{a}
Rockdust + N	0.072^{b}	0.23^{ab}	0.026^{b}	1.1^{g}	0.14^{bcde}	69^{ab}	0.020	0.78	2.8^{abc}	5.2^{abcd}
Wood ash	0.074^{b}	0.18^{bcde}	0.030^{b}	1.2^{defg}	0.099^{f}	59^{b}	0.020	0.74	2.4^{abc}	4.4^{bcdef}
Wood ash + N	0.074^{b}	0.19^{bcd}	0.028^{b}	1.3^{cdefg}	0.100^{f}	60^{b}	0.017	0.69	2.3^{abc}	4.2^{bcdef}

*p < 0.05; **p < 0.01; ***p < 0.001.
Note: ns, not significant.
Different letters within the same column show a significant difference as determined by ANOVA.

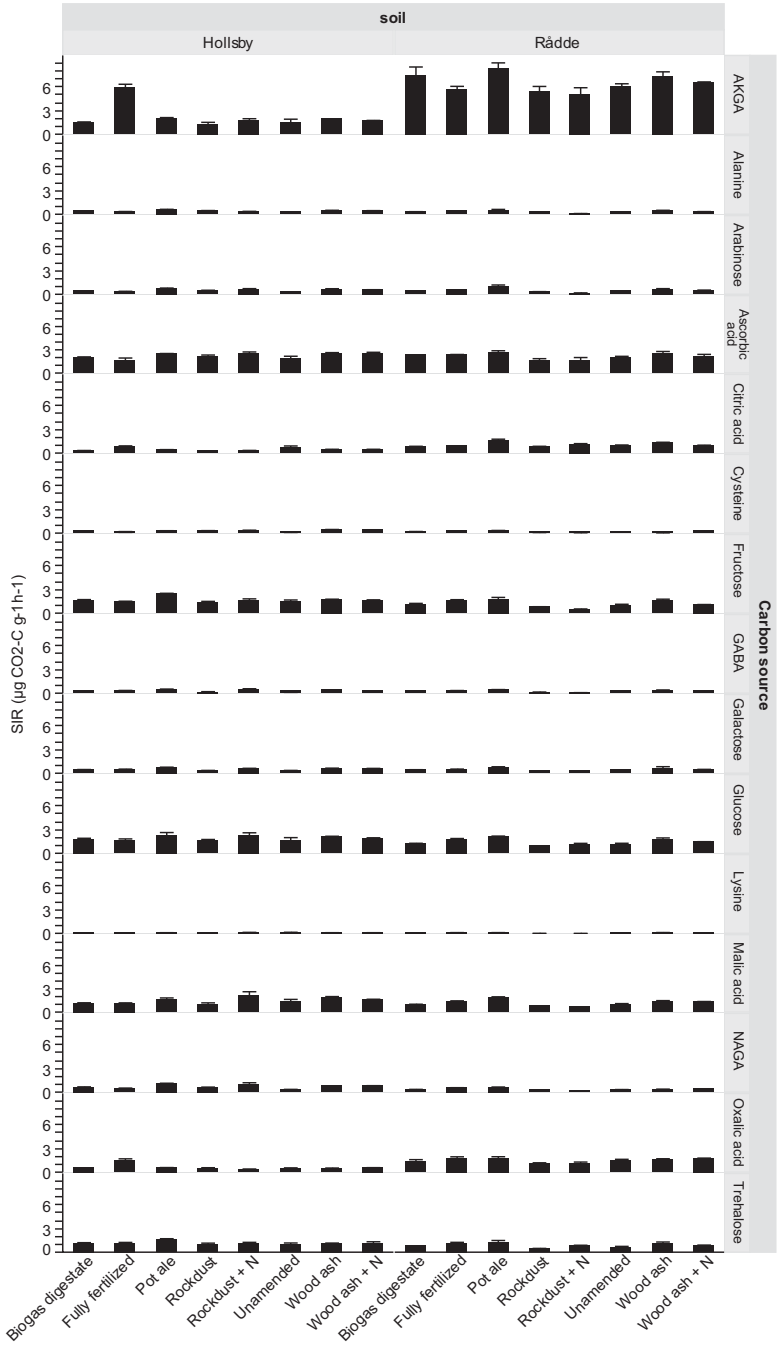

Figure 1. Substrate-induced respirations in different treatments based on each C source in soil samples 14 months after application (year 1). The error bars indicate standard errors. AKGA, α-ketoglutaric acid; GABA, γ-amino butyric acid; NAGA, N-acetyl-glucosamine; A., acid.

significantly different in pot ale and to a lesser extent biogas digestate treatments in Hollsby soil (year 1 only; Figure 3).

Molecular fingerprinting by T-RFLP of *Rhizobium/Agrobacterium* 16s rDNA detected 29 fragments with molecular weights ranging from 39 to 531 base pairs (bp), and the multivariate analysis was performed on the relative abundance data for the soil samples. The ordination diagram of the data showed that the greatest difference was once again between the two different soils, with CV 1 explaining 69% of the variation (Figure 5). T-RFLP results showed that the samples from the fully fertilised treatment of both soils were discriminated from all the other treatments of either soil (Figure 5). While it is not possible to attribute identities to all the fragments, the most abundant fragment (51Y) was most likely *R. leguminosarum* bv trifolii, as shown by testing a

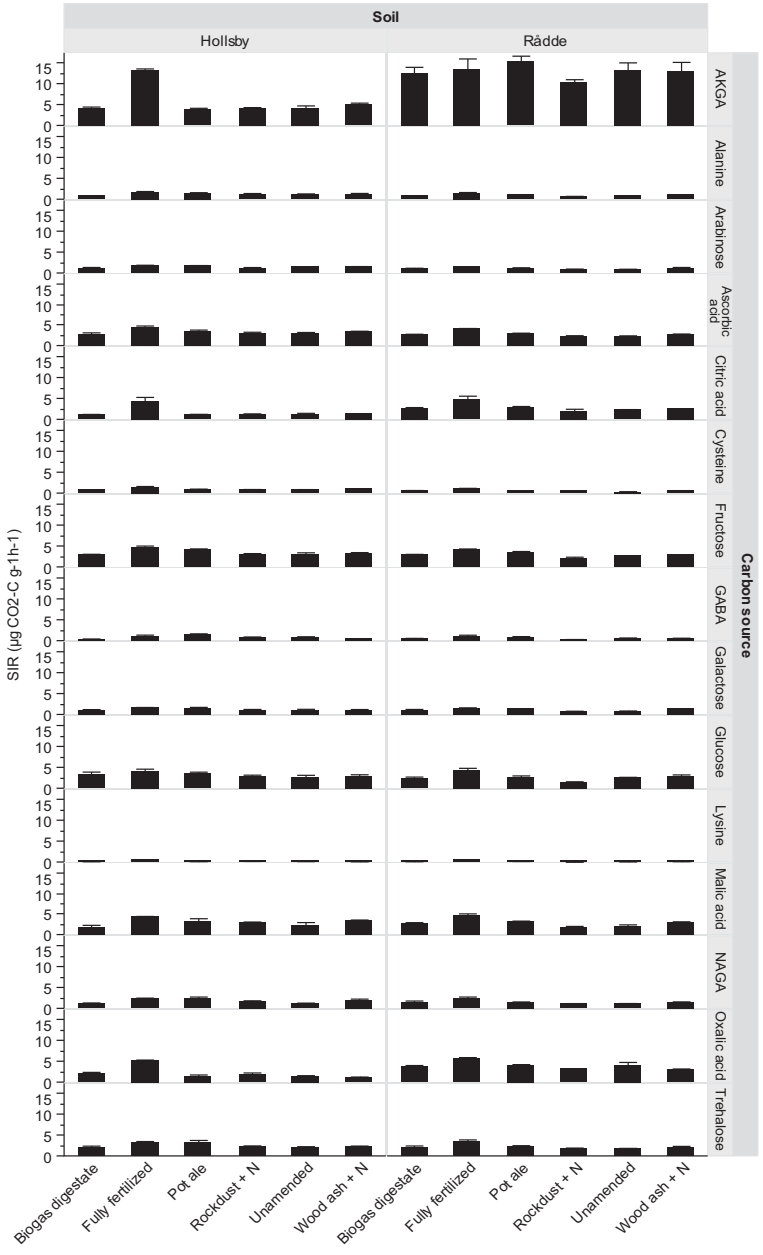

Figure 2. Substrate-induced respirations in different treatments based on each C source in soil samples 26 months after application (year 2). The error bars indicate standard errors. AKGA, α-ketoglutaric acid; GABA, γ-amino butyric acid; NAGA, N-acetyl-glucosamine; A., acid.

known culture as a positive control and which gave a single fragment with 100% relative abundance. This fragment was found in all the samples confirming that *R. leguminosarum* bv trifolii existed in all tested soil samples. In addition, there were another eight fragments with high relative abundance, i.e. above 90% averaged over all samples, and these are highlighted in Figure 6. The ordination of the loadings from the multivariate analysis showed one fragment (78Y) to be very different from the others as it had high positive ordinates on both the X and Y axis (Figure 6) while Fragment 51Y showed a tendency to occur at higher relative abundances in the fully fertilised treatment.

Plant biomass yield

The two soils included in the experiment produced significantly different yields of forage species, with Hollsby producing 1.3 and 1.2 times more than Rådde in years 1 and 2 respectively. The fully fertilised treatment produced the highest yield in both soils in both years. The increase compared to the unamended control was between 2.5 and 4.4 times more, and 5.3

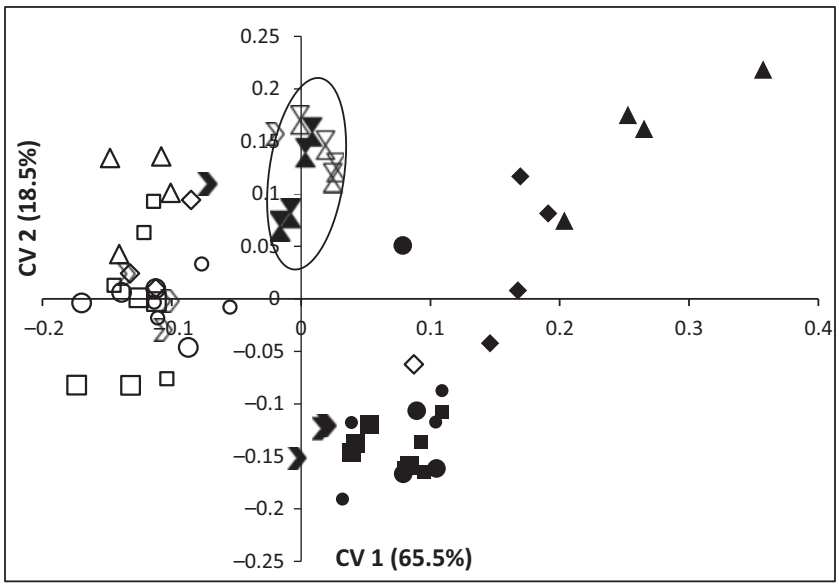

Figure 3. Ordination diagram of first and second CVs for MicroResp™ CLPPs with 15 C sources based on soil samples collected 14 months after application (year 1). Solid and empty symbols refer to Hollsby and Rådde soils respectively. The ellipse shows the cluster of fully fertilised treatments of both soils.
Note: ◇, Biogas digestate; ⟩⟩, unamended control; ⧖, fully fertilised; △, pot ale; ○, rockdust; ◯, rockdust + N; □, Wood ash; ▢, wood ash + N.

and 5.7 times more for Hollsby and Rådde respectively in years 1 and 2 (Table 7). In year 1, all amendments increased the biomass produced and led to distinctly different botanical compositions of the mixed crops (Table 7). In year 2, there were no significant differences in biomass production between the amended soils and the unamended controls, nor in the botanical composition of the crop.

Discussion

Characteristics of the soils and amendments

It is likely that the risks and benefits of different amendments will depend not only on their composition but also on the inherent properties of the soils to which they are applied. Here not only their chemical composition in terms of elements and key properties

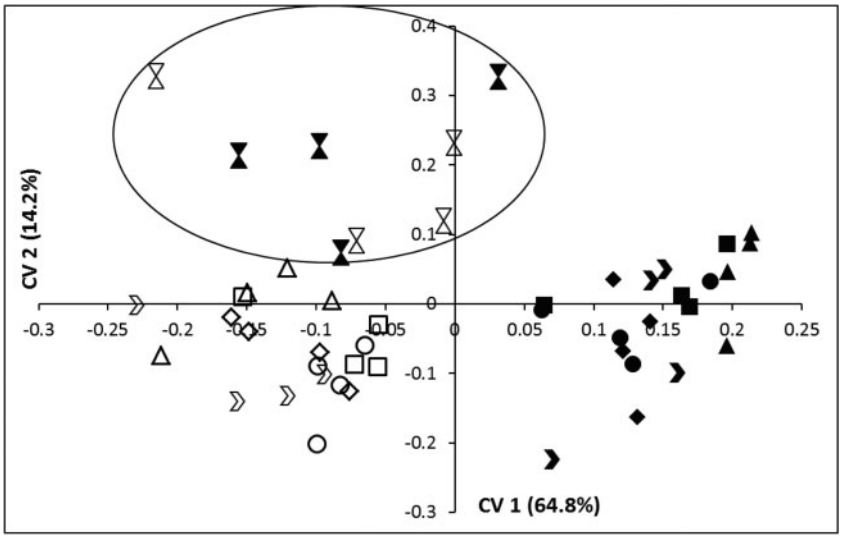

Figure 4. Ordination diagram of first and second CVs for MicroResp™ CLPPs with 15 C sources based on soil samples collected 26 months after application (year 2). Solid and empty symbols refer to Hollsby and Rådde soils respectively. Rockdust and wood ash (without N) were removed from the experiment in year 2. The ellipse shows the cluster of fully fertilised treatments of both soils.
Note: ◇, Biogas digestate; ⟩⟩, unamended control; ⧖, fully fertilised; △, pot ale; ◯, rockdust + N; □, wood ash + N.

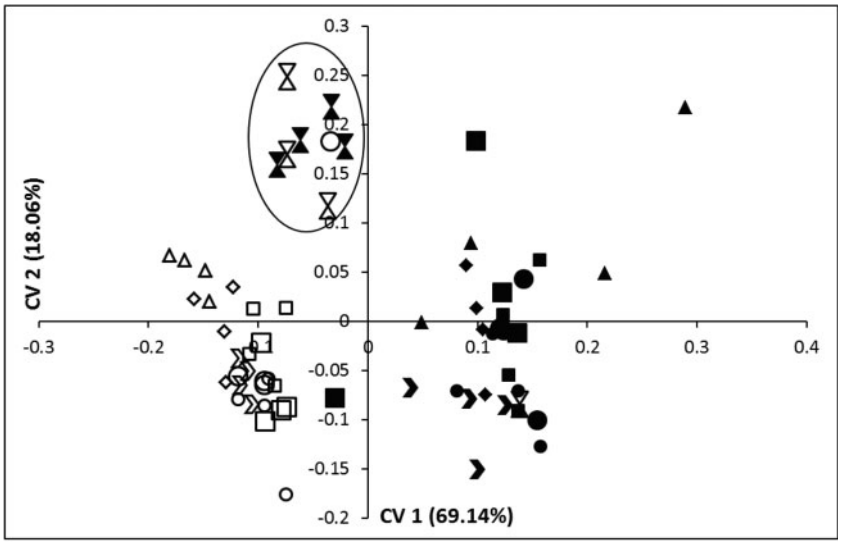

Figure 5. Ordination diagram of first and second CVs for T-RFLP data based on soil samples collected 14 months after application (year 1). Solid and empty symbols refer to Hollsby and Rådde soils respectively.
Note: ◇, Biogas digestate; ⟩⟩, unamended control; ⧖, fully fertilised; △, pot ale; ○, rockdust; ◯, rockdust + N; □, Wood ash; □, wood ash + N.

has been characterised, but also their mineralogical phases which helps to interpret their effects.

The mineralogical and chemical composition of a soil's parent material is reflected in the soil developed from it. In general, soils developed from sandstones and acid igneous rocks, such as granite, composed predominately of quartz and feldspar, tend to be lower in intrinsic nutritional elements inherited from the parent material compared to soils developed on mixed alluvial deposits and basic igneous rocks (He et al. 2005). Quantitative mineralogical analyses showed that approximately 80% of the minerals in both soils used in this experiment were quartz, K-feldspar and plagioclase (Table 3). These minerals weather slowly (Lasaga 1984), while more base-rich minerals such as amphibole and trioctahedral phyllosilicates (e.g. biotite, phlogopite and chlorite) are more easily weatherable. Smaller amounts of these base-rich minerals were found in both Rådde and Hollsby, but they may nevertheless have the potential

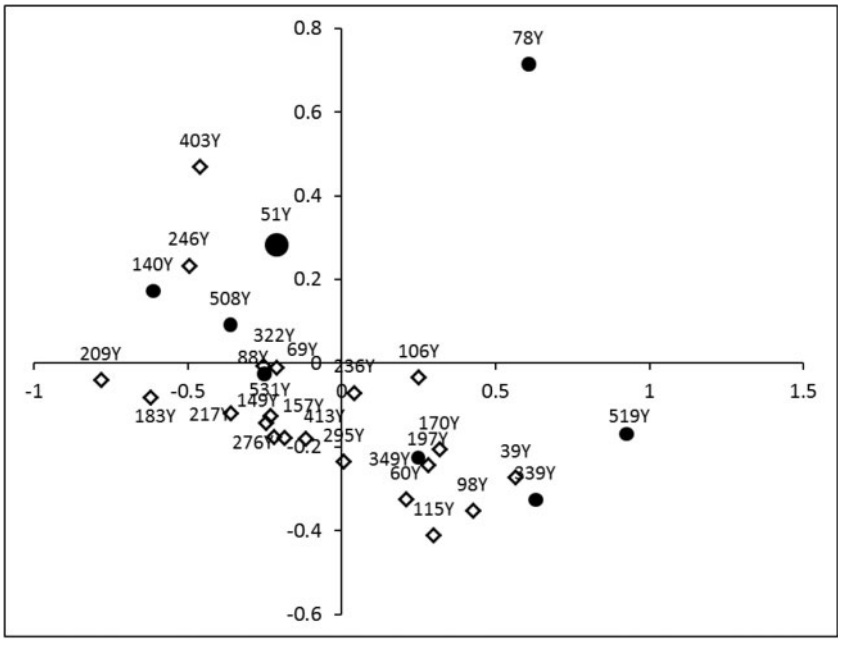

Figure 6. Ordination diagram of identified TRFs in the samples. The eight most abundant peaks are identified by black circles. Fragment 51Y represents *R. leguminosarum* bv trifolii.

Table 7. Cumulative yield and botanical composition (shown as clover proportion) of mixed stand of perennial ryegrass and red clover produced on two soils under different treatments (n = 4).

	Sum of harvest (g pot^{-1})		Clover proportion
	Year 1	Year 2	Year 1
Soils	**	*	**
Treatments	**	**	**
Soil × treatment	**	*	**
Hollsby			
Unamended	24^{def}	14^{bc}	0.7^{ab}
Fully fertilised	60^{a}	75^{a}	0.3^{de}
Biogas digestate	33^{b}	18^{b}	0.7^{ab}
Pot ale	30^{bc}	18^{b}	0.5^{c}
Rockdust	30^{bc}	–	0.8^{a}
Rockdust + N	29^{bcd}	14^{bc}	0.2^{e}
Wood ash	33^{b}	–	0.8^{a}
Wood ash + N	33^{b}	14^{bc}	0.3^{de}
Rådde			
Unamended	14^{g}	14^{bc}	0.5^{c}
Fully fertilised	62^{a}	80^{a}	0.3^{de}
Biogas digestate	26^{cde}	15^{bc}	0.6^{b}
Pot ale	20^{f}	10^{c}	0.3^{d}
Rockdust	20^{f}	–	0.6^{b}
Rockdust + N	22^{ef}	12^{bc}	0.5^{c}
Wood ash	26^{cde}	–	0.7^{ab}
Wood ash + N	28^{bcd}	12^{bc}	0.3^{de}

*p < 0.05; **p < 0.001.
Note: Different letters within the same column show a significant difference as determined by ANOVA.
Rockdust and wood ash were removed from the experiment in year 2.

to make relatively stronger contribution to the soil nutrient content by providing elements such as K (Andrist-Rangel et al. 2006) and Mg. In addition, the total amount of clay size particles was low in both soils, 4% and 8% in Hollsby and Rådde respectively, whereas Hollsby was dominated by silty material (69%) and Rådde by sand (62%; Table 1). Thus, the coarse soil texture and mineralogical composition confirm that the selected soils were low in base-rich and easily weatherable minerals, and therefore inherently limited in their capacity to supply essential elements for high biological production. Indeed, the two soils belonged to the 25% of Swedish soils with the lowest concentrations of Co, Cu, Mg, Ni and K. Hollsby in particular belonged to the 25% of Swedish soils with the lowest Cr and Mo concentration and Rådde with the lowest Zn concentration. Hence both soils, while different, were in theory likely to benefit from the nutrients in the different amendments. However, they also exhibited average to relatively high N_{tot}, P_{HCl} and S_{tot} (Table 1) for Swedish agricultural soils (Eriksson et al. 2010; Swedish monitoring program 2013). In the case of N, this is likely to be an effect of the high frequency of ley at Rådde and the permanent grassland at Hollsby. For P and S, it can probably be attributed to the history of P fertilisation and to S deposition (Larsson et al. 1995).

As with the soil, the benefit and behaviour of rockdust would also depend heavily on the mineralogical composition of the parent rock. Ramezanian et al. (2013) analysed the same rockdust used in this study and showed that it was dominated by plagioclase feldspar and contained some K-feldspar, along with some pyroxene, quartz and the iron and titanium oxides hematite and ilmenite. XRD analysis also showed the crystalline mineral phases that were present in other amenders. The wood ash mostly contained quartz as the crystalline phase, as found by Martins et al. (2007) as well, probably derived from contamination by sand and soil particles during harvest, transportation and combustion (Holmberg & Claesson 2001; Dahl et al. 2009). Portlandite and periclase were also found in the wood ash. This may be attributed to the transformation of carbonates during combustion (Holmberg & Claesson 2001), most likely forming calcium and magnesium oxides which may subsequently form hydroxides by hydration. The portlandite may also be a hydration product of the di-calcium silicate. These minerals undoubtedly contribute to the high liming effect of this amendment. In biogas digestate, apart from quartz and feldspars which can be related to soil particles as contaminants in the waste and silage, some struvite was found. Struvite is an orthophosphate-containing magnesium, ammonium and phosphate which has been found in various biological media such as cow manure and sheep faeces (Shand et al. 2005; Le Corre et al. 2009) and it also forms in wastewater plants from which it may (in principle) be recovered for use as a fertiliser (Booker et al. 1999; Le Corre et al. 2009). Nevertheless, since the amount of struvite was relatively low in the biogas digestate (judging from the XRD pattern a few weight percent is probable) and P was most likely not the limiting nutrient in the two soils, it is not surprising that the biogas digestate did not show a distinct P fertilising effect in the experiment.

Effect of the amendments on soil properties and extractable elements

As expected, wood ash significantly increased the pH of the soils after application due to its high liming effect (e.g. Park et al. 2012). Although the strong alkalinity of wood ash has to be considered in application rates, doses based on the lime requirements of most soils represent a negligible risk to the environment (Demeyer et al. 2001). At the applied rates, the ash increased soil pH_{H2O} and pH_{CaCl2} by 0.4 and 0.5 pH unit respectively compared to the unamended control. The somewhat larger increase

in soil pH in Hollsby than Rådde upon ash amendment when comparing the pH of the original and wood ash amended soils (Tables 2 and 4) might be due to the difference in soil texture with Hollsby dominated by silt and Rådde by sand (Table 1).

Wood ash increased the EDTA extractability of Ca, K and Mg in the soil compared with the other by-products, probably as a consequence of relatively large amounts added of these elements, combined with a high availability of these elements in the ash (Demeyer et al. 2001). The pH increase by the wood ash treatment could also have an effect since the solubility of Ca, K and Mg improves slightly when the pH increases from 5 to 6. Although the P concentration was also high in the wood ash, it did not increase the available P of the soils compared to other amendments, perhaps because this treatment added the least amount of P at the application rates chosen. While the wood ash application added considerable amounts of Mn, it did not increase the available Mn of the soils, probably as a consequence of the rise in pH which made the trace elements less available. For example, Mn^{2+} in soil solution decreases 100-fold for each unit increase in pH (Havlin et al. 1999). It has been confirmed that 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 soils. It may, however, decrease the availability of micronutrients due to the increase in pH, and this would be of concern in micronutrient-deficient soils.

Pot ale increased the Cu_{EDTA} in the soils, most likely due simply to the higher amount of Cu added to soils compared to all other treatments, as there were no other significant differences to basic soil properties which would have affected availability. This is in contrast with the results by Douglas et al. (2003) who reported no significant increase in soil Cu_{tot} after the application of pot ale in a field experiment. However, due to the large background variation in element composition, detecting changes in a soil's total concentration of an element often requires large increases. This may not be expected after only a few applications of pot ale if these are made in accordance with fertiliser N recommendations, as was the case in that study. In this study, the soils were deliberately chosen to reflect low background levels of many macro- and micronutrients and the EDTA extraction targets a smaller, and presumably more readily affected, fraction of the soil's Cu content.

While there is some evidence of the positive effects of rockdust on supplying crops with specific nutrients (e.g. Baerug 1991a; Bakken et al. 2000), there are other studies which find little or negligible benefit in the use of rockdust as a fertiliser (e.g. Bakken et al. 2000; Bolland & Baker 2000; Ramezanian et al. 2013). The potential of rockdust in providing plant nutrients is recognised to be strongly dependent on its mineralogy, but our results show it is also dependent on that of the soil and its general nutrient status. Ramezanian et al. (2013) tested the effect of the same rockdust used in this study on three soils of higher concentrations of most nutrients than the rockdust applied. In this study, the efficacy of rockdust was tested on nutrient-poor soils with lower soil pH, where rockdust increased plant biomass significantly compared to the unamended control in both soil types. However, this occurred in year 1 only suggesting that rockdust, although certainly not being a rapidly available nutrient source, may not necessarily supply more nutrients several years after application than during a first season, possibly due to a fresh surface effect. Mg_{EDTA} was also enhanced by rockdust application in both soils compared to the unamended control, although the concentration was still very low. The Mg concentration added may be related to its release from pyroxene and/or the trioctahedral clay minerals which are relatively abundant in the rock dust (Ramezanian et al. 2013) and the significant increase in the soil Mg_{EDTA} attributed to the much lower inherent/original Mg content of the soils in the current study, as well as the pH difference between the soils in this study (pH ~5.5) compared to the soils used by Ramezanian et al. (2013; pH ~6.5).

There was no significant increase in available Cd, Ni or Pb, despite the large application rates, especially in the rockdust treatment. Apart from reflecting that their concentrations were lower in the rockdust than in the soil, this also indicates that the elements were poorly soluble. For Cd and Ni, the concentrations in the wood ash were on the other hand distinctly higher than those of the soils. Available Cd and Ni still did not increase, probably because the application rate of wood ash was moderate and their availability in the soil limited due to the rise in pH.

Effects of the amendments on soil microbial communities

Soil microorganisms are good indicators for monitoring changes in soil quality due to the application of soil amendments. Although respiration from most of the individual substrates in our study was affected by amendments, there was no clear overall effect of amendments on the physiological profiles of the soil microbial community. There was, however, a clear change in the profile due to the fully fertilised treatment and indeed the profiles of both soils were very different in the unamended and amended soils but were indistinguishable in the fully fertilised

treatment between the soil types. This showed that the microbial communities in the two soils that were clearly different to begin with had both responded to the fully fertilised treatment in a similar manner and that the community composition had converged. This effect was constant during both years of the experiment (Figures 3 and 4). As there were two amendments (rockdust and wood ash) with and without N in the experiment, and there was no discrimination between them in either soil type in the ordination diagram, the effect of fully fertilised treatment on microbial communities was probably not due solely to N, but to the application of one or more other nutrients. That different plant species select different microbial communities and that increased plant growth stimulates microbial activity and growth is well known (Grayston et al. 1998; Grayston et al. 2004). Differences in microbial communities due to soil type differences are also often larger than management differences (Girvan et al. 2003) but in our study the fully fertilised treatment has overcome the differences in starting populations. This was not so clear for the other amendments. Of the two organic amendments, pot ale and to a lesser extent biogas digestate, showed some discrimination from the control in the Hollsby soil samples of year 1, but this was not seen in the Rådde soil (Figure 3). Microorganisms are generally C limited in soil and the size and activity of the microbial biomass is therefore proportional to the amount of available C in the soil (Marschner et al. 2003). Pot ale and biogas digestate added C_{org} to the soils. Given that Hollsby had lower C_{org} than Rådde, a slight alteration in Hollsby's microbial communities may have been more easily detected compared to those in Rådde. In a study by Lupwayi et al. (2009), principal component analysis on the SIR data of wood ash-treated soils with different crops showed detectable community structures according to the crop, but not between wood ash and control treatments which corroborates the lack of effect in our study. There are, however, other studies reporting that wood ash changed the substrate utilisation potential (Biolog method) of soil microbes (Liiri et al. 2001). Lack of any detectable effect on the CLPP obtained due to rockdust is in agreement with the study by Ramezanian et al. (2013) using the same rockdust on a different set of soils.

The primary coincident effect of the fully fertilised treatment in both soils was increased plant growth but this as deduced above was probably due to nutrients other than N alone. In year 1, the fully fertilised treatment produced the highest plant biomass in both soils, while it differed significantly among the amendments and the unamended control. However, in year 2, the plant biomass obtained from the fully fertilised treatment was still the highest by far, but there was no significant difference across all the amendments and the unamended control (Table 7). Further, there were significant differences in botanical composition between treatments in year 1 but not in year 2. Nevertheless, ordination diagrams based on MicroResp™ data show the same pattern in both years, indicating that the change in community response detected in the fully fertilised treatment was possibly a direct effect on the microbes of the nutrient addition and not only an effect of increased plant biomass growth or altered botanical composition. This can be corroborated by the fact that we could find no significant correlations between plant yield and SIR across all samples either year (data not shown). Further, although Dahlin et al. (2015) showed that in the same experiment there were botanical differences between these treatments and despite knowing variation in plant community can affect microbial community composition (Lupwayi et al. 2009), we did not see any significant differences. The ANOVA showed that microbes of the soils treated with pot ale had a higher SIR rate compared to other amendments, and so this organic amendment was also stimulating the microbial biomass. As there was no significant difference in the harvested plant biomass between the amendments in Hollsby and significantly less than some of other amendments in Rådde in year 1, this suggests the effect was due directly to the effect of pot ale on the microbial community, e.g. via the added C_{org}, as discussed earlier.

Figure 5 indicates that the *Rhizobium/Agrobacterium* community, regardless of soil differences, was affected by the availability of nutrients provided through the fully fertilised treatment. This is quite surprising as there have been few studies that have shown such an effect in (micro)nutrient-deficient soils, suggesting that some of the species in this microbial community are also nutrient limited. The ordination diagram (Figure 6) showed that the fragments which were most likely to be representing *Rhizobium/Agrobacterium* were scattered across the different treatments and were not clustered with any single soil amendment. Thus the *Rhizobium/Agrobacterium* genotypes were not significantly altered by the amendments. Consistent with this, the most abundant fragment, 51Y – a presumptive *R. leguminosarum* – had the highest abundance in the fully fertilised soil samples (Figure 6).

In an incubation study, Söderström (2012) found that addition of single macronutrients and micronutrients to the same soils (Rådde and Hollsby) without plant inputs did also 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 by Mg, K and N, supporting the original hypothesis concerning nutrient limitation of microbes and plants. It follows that mineral nutrients may 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).

While we have shown in practice negligible risks of application of the tested by-products at the used rates it is clearly important to test further the effect of repeated amendments and the long-term effects and even combinations of different amendments (Ray et al. 2013) to get optimal matches between the beneficial constituents and the requirements of different soils. Since different types of by-products which might have the potential to be used as soil amendments have their own special characteristics, it is recommended that soil and by-product benefits are matched to optimise efficiency in their usage.

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Disclosure statement

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Supplemental data

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References

Abubaker J, Risberg K, Jönsson E, Dahlin AS, Cederlund H, Pell M. Forthcoming 2015. Short-term effects of biogas digestates and pig slurry application on soil microbial activity. Appl Environ Soil Sci. Available from: http://www.hindawi.com/journals/aess/2015/658542/

Agbede TM, Adekiya AO. 2012. Effect of wood ash, poultry manure and NPK fertilizer on soil and leaf nutrient composition, growth and yield of okra (*Abelmoschus esculentus*). Emir J Food Agric. 24:314–321.

Anderson MJ, Willis TJ. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology. 84:511–525.

Andrist-Rangel Y, Simonsson M, Andersson S, Oborn I, Hillier S. 2006. Mineralogical budgeting of potassium in soil: a basis for understanding standard measures of reserve potassium. J Plant Nutr Soil Sci. 169:605–615.

Arthurson V. 2009. Closing the global energy and nutrient cycles through application of biogas residue to agricultural land – potential benefits and drawbacks. Energies. 2:226–242.

Baerug R. 1991a. Rock powder as a source of nutrients to different crops the effect of potassium in rock powder. Norsk Landbruksforsking. 5:175–182.

Baerug R. 1991b. Rock powder as a source of nutrients to different crops the magnesium effect of rock powder. Norsk Landbruksforsking. 5:183–188.

Bakken AK, Gautneb H, Sveistrup T, Myhr K. 2000. Crushed rocks and mine tailings applied as K fertilizers on grassland. Nutr Cycl Agroecosyst. 56:53–57.

Berthelet M, Whyte LG, Greer CW. 1996. Rapid, direct extraction of DNA from soils for PCR analysis using polyvinylpolypyrrolidone spin columns. Fems Microbiol Lett. 138:17–22.

Bolland MDA, Baker MJ. 2000. Powdered granite is not an effective fertilizer for clover and wheat in sandy soils from Western Australia. Nutr Cycl Agroecosyst. 56:59–68.

Booker NA, Priestley AJ, Fraser IH. 1999. Struvite formation in wastewater treatment plants: opportunities for nutrient recovery. Environ Technol. 20:777–782.

Bucknall SA, McKelvie AD, Naylor REL. 1979. Effects of application of distillery pot ale to hill vegetation and lowland crops. Ann Appl Biol. 93:67–75.

Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM. 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl Environ Microbiol. 69:3593–3599.

Campbell NS. 2009. The use of rockdust and composted materials as soil fertility amendments [dissertation]. Glasgow: University of Glasgow.

Chaudri AM, McGrath SP, Giller KE, Rietz E, Sauerbeck DR. 1993. Enumeration of indigenous *Rhizobium leguminosarum* biovar Trifolii in soils previously treated with metal-contaminated sewage-sludge. Soil Biol Biochem. 25:301–309.

Coroneos C, Hinsinger P, Gilkes RJ. 1996. Granite powder as a source of potassium for plants: a glasshouse bioassay comparing two pasture species. Fertil Res. 45:143–152.

Dahl O, Nurmesniemi H, Poykio R, Watkins G. 2009. Comparison of the characteristics of bottom ash and fly ash from a medium-size (32 MW) municipal district heating plant incinerating forest residues and peat in a fluidized-bed boiler. Fuel Process Technol. 90:871–878.

Dahlin AS, Ramezanian A, Campbell CD, Hillier S, Öborn I. 2015. Waste recovered by-products can increase growth of grass-clover mixtures in low fertility soils and alter botanical and mineral nutrient composition. Ann Appl Biol. 166:105–117.

Demeyer A, Nkana JC, Verloo MG. 2001. Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. Bioresour Technol. 77:287–295.

Douglas JT, Aitken MN, Smith CA. 2003. Effects of five non-agricultural organic wastes on soil composition, and on the yield and nitrogen recovery of Italian ryegrass. Soil Use Manage. 19:135–138.

Erich MS. 1991. Agronomic effectiveness of wood ash as a source of phosphorus and potassium. J Environ Qual. 20:576–581.

Eriksson J, Mattsson L, Söderström M. 2010. Tillståndet i svensk åkermark och gröda, 584 data från 2001–2007, 585 [Current status of Swedish arable soils and cereal crops. Data from the period 2001–2007]. Swedish.

Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS. 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol. 69:1800–1809.

Grayston SJ, Campbell CD, Bardgett RD, Mawdsley JL, Clegg CD, Ritz K, Griffiths BS, Rodwell JS, Edwards SJ, Davies WJ, et al. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Appl Soil Ecol. 25:63–84.

Grayston SJ, Wang SQ, Campbell CD, Edwards AC. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem. 30:369–378.

Haraldsen TK, Andersen U, Krogstad T, Sorheim R. 2011. Liquid digestate from anaerobic treatment of source-separated household waste as fertilizer to barley. Waste Manag Res. 29:1271–1276.

Havlin JL, Beaton JD, Tisdale SL, Nelson WL. 1999. Soil fertility and fertilizers, an introduction to nutrient management. Upper Saddle River (NJ): Prentice Hall.

He ZL, Yang XE, Stoffella PJ. 2005. Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol. 19:125–140.

Hillier S. 1999. Use of an air brush to spray dry samples for X-ray powder diffraction. Clay Minerals. 34:127–135.

Holmberg SL, Claesson T. 2001. Mineralogy of granulated wood ash from a heat plant in Kalmar, Sweden. Environ Geol. 40:820–828.

Huang H, Campbell AG, Folk R, Mahler RL. 1992. Wood ash as a soil additive and liming agent for wheat – field studies. Commun Soil Sci Plant Anal. 23:25–33.

KLS. 1965. Kungliga Lantbruksstyrelsens kungörelse med (5) bestämmelser för undersökning av jord vid statens lantbrukskemiska kontrollanstalt och lantbrukskemisk kontrollstation och lantbrukskemisk station med av staten fastställda stadgar. Kungliga Lantbruksstyrelsens kungörelser m.m., Nr 1 [Royal Agricultural Board announcement with 5 regulations for analysis of soil at the State Institute for Agricultural Chemistry and Laboratories for Agricultural Chemistry according to state-established statutes]. Swedish.

Larsson E, Knulst J, Malm G, Westling O. 1995. Deposition of acidifying compounds in Sweden. Water Air Soil Pollut. 85:2271–2276.

Lasaga AC. 1984. Chemical-kinetics of water-rock interactions. J Geophys Res. 89:4009–4025.

Le Corre KS, Valsami-Jones E, Hobbs P, Parsons SA. 2009. Phosphorus recovery from wastewater by struvite crystallization: a review. Crit Rev Environ Sci Technol. 39:433–477.

Liiri M, Setala H, Haimi J, Pennanen T, Fritze H. 2001. Influence of *Cognettia sphagnetorum* (Enchytraeidae) on birch growth and microbial activity, composition and biomass in soil with or without wood ash. Biol Fertil Soils. 34:185–195.

Losak T, Hlušek J, Zatloukalová A, Musilová L, Vítězová M, Škarpa P, Zlámalová T, Fryč J, Vítěz T, Mareček J, Mårtensson A. 2014. Digestate from biogas plants is an attractive alternative to mineral fertilisation of Kohlrabi. J Sustain Dev Energy Water Environ Syst. 4:309–318.

Lupwayi NZ, Arshad MA, Azooz RH, Soon YK. 2009. Soil microbial response to wood ash or lime applied annual crops and perennial grass in an acid soil of northwestern Alberta. Can J Soil Sci. 89:169–177.

Marschner P, Kandeler E, Marschner B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol Biochem. 35:453–461.

Martins FM, Martins JM, Ferracin LC, da Cunha CJ. 2007. Mineral phases of green liquor dregs, slaker grits, lime mud and wood ash of a Kraft pulp and paper mill. J Hazard Mater. 147:610–617.

Omotoso O, McCarty DK, Hillier S, Kleeberg R. 2006. Some successful approaches to quantitative mineral analysis as revealed by the 3rd Reynolds Cup contest. Clays Clay Minerals. 54:748–760.

Park ND, Rutherford PM, Thring RW, Helle SS. 2012. Wood pellet fly ash and bottom ash as an effective liming agent and nutrient source for rye grass (*Lolium perenne* L.) and oats (*Avena sativa*). Chemosphere. 86:427–432.

Pitman RM. 2006. Wood ash use in forestry – a review of the environmental impacts. Forestry. 79:563–588.

Ramezanian A, Dahlin AS, Campbell CD, Hillier S, Mannerstedt-Fogelfors B, Öborn I. 2013. Addition of a volcanic rockdust to soils has no observable effects on plant yield and nutrient status or on soil microbial activity. Plant Soil. 367:419–436.

Ray SNC, Yadav UP, Prasad BL, Agarwal PK, Singh S, Sinha RC. 2013. Efficacy of a mix of fly-ash and post methanated diluted distillery effluent as a potential biofertilizer: a case study on the biomass of black mustard, *Brassica nigra*. Int J Res Chem Environ. 3:153–160.

Reverchon F, Xu Z, Blumfield TJ, Chen C, Abdullah KM. 2012. Impact of global climate change and fire on the occurrence and function of understorey legumes in forest ecosystems. J Soils Sediment. 12:150–160.

Ritz K, Black HIJ, Campbell CD, Harris JA, Wood C. 2009. Selecting biological indicators for monitoring soils: a framework for balancing scientific and

technical opinion to assist policy development. Ecol Indicat. 9:1212–1221.

Schloter M, Dilly O, Munch JC. 2003. Indicators for evaluating soil quality. Agric Ecosyst Environ. 98: 255–262.

Shand CA, Coutts G, Hillier S, Lumsdon DG, Chudek A. 2005. Phosphorus composition of sheep feces and changes in the field determined by P-31 NMR spectroscopy and XRPD. Environ Sci Technol. 39:9205–9210.

Singh BK, Nazaries L, Munro S, Anderson IC, Campbell CD. 2006. Use of multiplex terminal restriction fragment length polymorphism for rapid and simultaneous analysis of different components of the soil microbial community. Appl Environ Microbiol. 72:7278–7285.

Söderström K. 2012. Nutrient effects on microorganism communities in two nutrient poor soils [MSc. report]. Uppsala: Swedish University of Agricultural Sciences.

Streck T, Richter J. 1997. Heavy metal displacement in a sandy soil at the field scale.1. Measurements and parameterization of sorption. J Environ Qual. 26:49–56.

Sumner ME. 1994. Measurement of soil-ph – problems and solutions. Commun Soil Sci Plant Anal. 25:859–879.

Svensson K, Odlare M, Pell M. 2004. The fertilizing effect of compost and biogas residues from source separated household waste. J Agric Sci. 142:461–467.

Swedish Board of Agriculture [Internet]. 2011. Föreskrifter om ändring i Statens jordbruksverks föreskrifter och allmänna råd (SJVFS 2004:62) om miljöhänsyn i jordbruket vad avser växtnäring [Amendment to the regulations and recommendations of the Swedish Board of Agriculture (SJVFS 2004:62) on environmental considerations regarding plant nutrients.] [cited 2013 Jan 12] Available from: http://www.jordbruksverket.se/download/18.53b6e8e714255ed1fcc52c0/1385727685022/2013-040.pdf.Swedish

Swedish Environmental Protection Agency (SEPA) [internet]. 1994. Statens Naturvårdsverks författningssamling [Statutes of the Swedish Environmental Protection Agency]. [cited 2013, Jan 12]. Available from: http://www.naturvardsverket.se/Documents/foreskrifter/nfs1994/SNFS1994_02k.pdf

Swedish Monitoring Program [Internet]. 2013. Swedish environmental monitoring program on arable soils. [cited 2013, Jan 12] Available from: http://www-jordbruksmark.slu.se/AkerWebb/MgiPub/Index.jsp?PageType=3&PageID=0.Swedish

Vincent JM. 1982. Nitrogen fixation in legumes. London (UK): Academic press.

Yunusa IAM, Loganathan P, Nissanka SP, Manoharan V, Burchett MD, Skilbeck CG, Eamus D. 2012. Application of coal fly ash in agriculture: a strategic perspective. Crit Rev Environ Sci Technol. 42: 559–600.