Organic Residues

A Resource for Arable Soils

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Abstract

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An increased recirculation of urban organic residues to arable soils has several environmental benefits, but there is a need for reliable test systems to ensure that soil quality is maintained. In this thesis, soil microbial, chemical and physical properties were included in an integrated evaluation to reflect the positive and negative effects of amending arable soils with organic residues.

Efficient statistical tools and methods to describe intrinsic spatial variation are important when evaluating soil data. A new method was developed, combining near infrared reflectance (NIR) spectroscopy with principal component analysis (PCA). The first principal component (PC1) of NIR data described spatial soil variation better than the conventional soil variables total carbon, clay content and pH.

A long-term field trial was established in which the soil was amended annually with organic residues (compost, biogas residues, sewage sludge) and fertilizers (pig manure, cow manure and mineral fertilizer, NPS). Annual measurements of soil and crop quality as well as yield revealed that biogas residues performed best among the organic residues. It improved several important microbiological properties, such as substrate-induced respiration (SIR) and potential ammonium oxidation (PAO), and it compared well with mineral fertilizer in terms of grain quality and harvest yield. Altogether, the results from the field trial showed no negative effects from any of the organic residues.

Short- and moderately long-term effects of wood ash and compost on potential denitrification activity (PDA) and PAO were evaluated in a laboratory incubation experiment. Wood ash application had a profound toxic effect on PDA both in the short- and long-term. This toxic effect was mitigated when compost was added to the soil.

Keywords: biogas residues, compost, field experiment, geostatistics, NIR, PCA, sewage sludge, soil microbiology, spatial variation, wood ash

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Appendix

Papers I-IV

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Odlare, M., Svensson, K. & Pell, M. 2005. Near infrared reflectance spectroscopy for assessment of spatial soil variation in an agricultural field. Geoderma 126, 193-202.

II. 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.

III. Odlare, M., Pell, M. & Svensson, K. 2005. Changes in soil chemical and microbiological properties after application of compost, biogas residues and sewage sludge – a field experiment. (Manuscript)

IV. Odlare, M. & Pell, M. 2005. Effect of wood ash and compost on nitrification and denitrification in soil. (Manuscript)

The author's contribution to the papers has been as follows:

I. Major part of the planning, data evaluation and writing of the manuscript. Performed minor part of the laboratory work.

II. Participated in planning and writing of the manuscript.

III. Major part of the planning, data evaluation and writing of the manuscript. Performed minor part of the laboratory work.

IV. Major part of the planning, data evaluation and writing of the manuscript. Performed most of the laboratory work apart from the PDA-analysis.

Introduction

Background

In the industrialized countries, natural resources are being consumed at an increasing rate, and solving the problem of the waste produced has become a major environmental challenge. Indeed, the EU's Sixth Environment Action Programme identifies waste prevention and waste management as one of four top priorities. In 1995, roughly 200 million tonnes of municipal solid waste was produced in the EU, and nearly half this volume was biodegradable organic waste. The huge volume and the increasing cost and environmental impact have prompted the implementation of EU policies to markedly reduce the amount of organic waste deposited in landfills. Organic waste can be transformed into a valuable and beneficial source of plant nutrients and soil conditioner that should preferably be used in agriculture and horticulture. The environmental policy of the European Commission has introduced several instruments to regulate the management of organic waste (Landfill Directive 1999/31/EC, Sewage Sludge Directive 86/278/EEC and organic farming regulation (EEC) No. 2092/91). In Sweden, deposition of organic waste in landfills was prohibited from January 2005.

Farmers and gardeners have long recognized the importance of replacing nutrients and organic matter that may be depleted under continuous cropping. Renewed and growing interest in "nutrient cycling" can be attributed to high costs of mineral fertilizers and the increasing need for suitable disposal of organic wastes. Through agricultural utilization of organic wastes, producers can benefit (and possibly derive marketing potential) from materials that otherwise would be placed in landfills causing environmental pollution problems.

Production of organic residues

When organic waste, such as food and garden litter, is subject to biological treatment the remaining product can be referred to as organic residue (Box 1). The organic residue should no longer be considered as a waste product, but rather a resource that should be manufactured and utilized in the best possible way. In this thesis, three main types of organic residues are discussed: compost from source-separated household waste, biogas residues from source-separated household waste and sewage sludge from wastewater treatment plants. Most organic substrates carry an indigenous population of microbes from the environment. The microbes of importance for treatment of organic material represent two major groups: *bacteria* (including *actinomycetes*) and *fungi*. The various subpopulations participate in the different phases of aerobic or anaerobic treatment processes, and consequently the overall microbial population changes.

Composting is an aerobic, self-heated decomposition and transformation of organic material. The decomposition proceeds from a mesophilic phase into a thermophilic phase and then through a gradual cooling and stabilization phase. Due to the high temperature in the thermophilic phase, the end-product, compost, becomes hygienic and weed seeds lose their ability to germinate. In 2003, 28 large-scale municipal composting plants were operating in Sweden, producing almost 140 000 tonnes of compost. Biogas residue is a by-product of the anaerobic digestion process of organic material in which biogas is extracted. During anaerobic digestion, complex organic material is sequentially subject to hydrolysis, fermentation and finally methanogenesis, where a large part of the energy contained in the organic material is conserved in methane. In 2003, 12 large-scale biogas plants in Sweden produced nearly 217 000 tonnes of biogas residue. Only a few of the biogas plants in Sweden use source-separated household waste as the main substrate. The remainder use mainly animal manure and industrial organic waste. Sewage sludge is an organic residue formed in the activated sludge from the wastewater treatment process. First, aerobic treatment degrades the organic material and growing microorganisms together with the organic residue form flocks that settle to the bottom. The surplus sludge is then subject to anaerobic digestion that reduces the volume and makes the product more hygienic. In Sweden, 240 000 tonnes of dewatered sewage sludge is produced yearly in municipal treatment plants.

Box 1. Definitions used in this thesis.

Organic waste	Societal organic waste products, e.g. food waste, restaurant waste, garden waste.
Organic residues	Organic waste that has been transformed into a new product after being subject to aerobic or anaerobic biological treatment, e.g. biogas residues, compost and sewage sludge.
Organic fertilizer	Organic material with sufficient amounts of plant nutrients e.g. organic residues, cow manure and pig slurry.

Use of organic residues in agriculture

The use of organic residues is not novel. Historical records show that the ancient Greeks and Romans were familiar with the decay processes that occurred spontaneously in stacks of plant matter or animal wastes. Soil has been amended with compost made from garden waste for centuries, but in more recent years organic residues from source-separated household waste and wastewater treatment have gained more attention. All three organic residues contain substantial amounts of organic material which, when added to the soil, will increase the humus content, increase the water holding capacity and improve soil structure. A good soil structure is important for root penetration, and for adequate drainage and aeration. In addition to organic material, organic residues contain several important plant nutrients such as N, P, K and Mg. Amendment with organic matter will also increase microbial activity in the soil, which in turn improves nutrient availability to the roots.

Compost

Compost from source-separated household waste contains relatively high amounts of plant available P and several studies have shown that compost application significantly increases available P in the soil (Steffens *et al.*, 1996; Boisch *et al.*, 1997; Ebertseder, 1997; Richter *et al.*, 1997). Compost also contains significant amounts of available K and the entire plant need of K can probably be provided by compost. Due to a relatively low concentration of mineral N in compost, compost application is not likely to increase the crop yield in the short term. Immediately after application, NH_4^+ is probably immobilized into microorganisms and is therefore not available to the plants. Eventually, an organically-bound N pool starts to build up. This mineralizable N-pool is in equilibrium with NH_4^+ (Fig. 1) and the release of NH_4^+ is determined by the C:N-ratio in the soil. Initially, after only a short period of compost application, the reaction to the right is very slow and only small amounts of $NH_4^+$are mineralized.

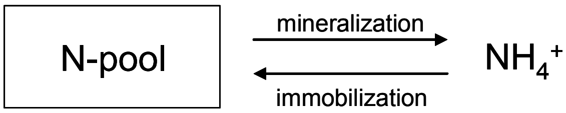

Figure. 1. A schematic figure of the equilibrium between the N-pool and NH_4^+-N in a compost fertilized soil.

However, after several years of application the N-pool will have increased to the point where the reaction is pushed to the right and consequently, organic N is mineralized to NH_4^+, thereby becoming available to plants. Then it may be possible to observe an effect of compost application on crop yields. On light soils, there is a potential risk for leaching due to nitrification and some studies suggest that compost should be applied frequently in small doses instead of large occasional ones (Poletschny, 1994; Werner *et al.*, 1998). In addition to plant nutrient supply, compost has been reported to improve soil structure (Jakobsen, 1995), increase enzyme activity (Perucci, 1990) and improve disease resistance (Hoitink & Boehm, 1990; Pascual *et al.*, 2000). Household waste is frequently mixed with about 30% urban park and garden waste in order to improve its structure and increase the C:N-ratio. Due to anthropogenic activities, such as leaded fuel in the past, this park and garden waste often contain a substantial amount of heavy metals which may lead to increased amounts of heavy metals in the compost. On the other hand, compost has been proven to reduce the phytoavailability of heavy metals in the soil (Brown *et al.,* 2004) and may therefore serve as a metal stabilizing agent.

Biogas residues

Compared to compost, the use of biogas residues in agriculture is relatively new. In some parts of Europe, on-farm digestion of organic material is common practice, whereas in Sweden, large-scale digestion plants utilizing source separated household waste are more common. Biogas residue is nutrient rich. All plant nutrients such as N, P, K and Mg, as well as trace elements essential to the plant, are preserved in the residue. One of the major advantages of biogas residues is the high content of NH_4^+-N, which when added to the soil is immediately available to plants. The residue has a high water content (95-98%), which makes it expensive to handle and to spread in the field. On drying, as much as 90% of the NH_4^+ can be lost as NH_3 (Rivard *et al.,* 1995). Biogas residues from source-separated household waste normally contain very low concentrations of heavy metals. This is because biogas residue is not mixed with park and garden waste. Research on the use of biogas residues in agriculture has not been as extensive as that on other organic wastes. However, several studies show that biogas residues can increase crop yield (Rivard *et al.,* 1995; Tiwari *et al.,* 2000).

Sewage sludge

The question as to whether agricultural soils should be amended with sewage sludge or not has been debated since the late 1960s. Alarming reports on high concentrations of heavy metals and organic pollutants have made both farmers and consumers suspicious of the use of sewage sludge in food cropping systems. Sewage sludge was commonly dumped in the sea until 1998 when this was prohibited by EU law (Urban waste water treatment directive 91/271/EEC). Production of sewage sludge continues to grow and, needless to say, the problem of how to handle this waste product urgently needs resolving. To address this question, much research has focused on sewage sludge application in agriculture and knowledge in this field is constantly growing. Sewage sludge contains a significant amount of P as a result of the biological and chemical precipitation of P in the wastewater treatment process. This nutrient pool is not always available for crops due to strong P sorption by Fe-oxides. However, several studies have shown that the amount of available P in the soil increased after sludge application (Johansson *et al.,* 1999; Debosz *et al.,* 2002). Bioavailable heavy metals are known to be toxic to microorganisms. Therefore, soils treated with metal contaminated sludge could adversely affect the microbes and hence the nutrient cycling in the soil sludge-plant system. However, Chander *et al.* (1995) and Johansson *et al.* (1999) concluded that none of the metals investigated in their studies showed any adverse effects on soil microbiology.

Wood ash

In addition to the organic residues mentioned above, about 300 000 tonnes of wood ash, a by-product from the incineration process in thermal power plants, is produced in Sweden annually. Wood ash is a concentrated form of the elemental constituents in the wood, with the exception of C and N, which are mostly volatilized during the incineration. It has alkaline properties (Etiegni *et al.,* 1991b; Jacobson *et al.,* 2004) and is therefore frequently used as a liming agent in acid soils. In addition, wood ash contains salts, oxides and hydroxides of Ca, K, Fe, Al, Mn, Na, Mg and other trace elements in smaller concentrations (Pepin & Coleman, 1984) and it has been shown to increase crop production (Meyers & Kopecky, 1998; Patterson *et al.,* 2004). However, due to anthropogenic activities, forests are subject to deposition of heavy metals, so that there is risk for high concentrations of heavy metals in the wood ash.

Significance of field-scale studies

During the last three decades, numerous scientific studies have generated a large body of information on the environmental effects and benefits associated with land application of organic residues (Bastian, 2005). More than 2000 technical papers have been published regarding land application of organic residues alone (O'Connor *et al.,* 2005). Much of this research has been conducted at the laboratory or greenhouse scale, where environmental conditions are controlled. However, results from laboratory and greenhouse conditions often cannot be extrapolated to field conditions. Although field studies inherently involve high cost and the risk of failure for reasons beyond the control of the researcher, they offer the ultimate scenario for addressing research questions. Long-term studies are particularly valuable to document the sustainability of application practices and to provide information on long-term environmental effects. Moreover, in order to convince suspicious farmers of the good in using organic residues, there is a need for reliable test systems, and long-term field experiments are probably the only way.

Soil microbes as sensitive probes

The important role that microorganisms play in the nutrient and energy flows in natural and man-manipulated environments implies the need for easily measured biological indicators of ecosystem disturbance. Microbes are in close contact with all three soil phases (solid, water and air) and therefore, they ought to respond rapidly and sensitively to changes in the soil environment (Kennedy & Papendick, 1995). Soil quality is a broad concept that has been defined in many ways. Doran & Parkin (1994) defined soil quality as: "the capacity of a soil to sustain biological productivity, maintain environmental quality, and promote plant and animal health", whereas Gregorisch *et al.* (1994) simply stated that soil quality is "the degree of fitness of a soil for a specific use". Karlen *et al.* (1998) concluded that it is not possible to measure soil quality directly, and therefore, soil quality indicators should be used instead. Although the concept of soil quality has been disputed (Sojka & Upchurch, 1999), it can be a useful analytical framework when assessing changes in the soil environment over a period of time. Microbial soil variables can be seen to serve two purposes: firstly; they are valuable and interpretable themselves and secondly; they can function as indicators of soil changes that can be difficult to measure in other ways. The origin and mechanisms of these soil changes are not always fully known. Therefore, the concept of soil quality can be used as a 'Black Box' to describe these unknown soil changes. If the content of the black box is determined by several complex soil physical, chemical and biological variables, then the choice of methods to evaluate this Black Box is dependent on the specific purpose of the soil. For example, from the farmer's point of view, crop yield may be the most important indicator of soil quality, while reduction in heavy metal content may be more important for someone dealing with bioremediation.

This thesis deals with changes in soil quality after amending the soil with different organic residues. Many of the existing methods used to evaluate treatment effects on soil quality focus mostly on soil chemical properties which change slowly with time, such as C, N and P content, which limits their usefulness for detecting short-term changes in soil quality. Instead, measurements of the status and activity of specific microbial communities contributing to soil processes should be performed. They have the potential to provide particularly rapid and sensitive means of characterizing changes in soil quality. Among the soil biological properties that have been suggested as indicators of soil quality are microbial biomass (Doran & Parkin, 1994; Boehm & Anderson, 1997; Sparling, 1997), enzyme activity (Helgason *et al.,* 1998; Badiane *et al.,* 2001; Chang *et al.,* 2001b) and composition of the microbial flora (Jordan *et al.,* 1995; Perkins & Kennedy, 1996). Microbial activity reflects the effects of both the living cell *per se* (the microbiological perspective) and nutrient cycling (the farmer's perspective).

Importance of statistical methods

Knowledge of the spatial variation of soil properties is important in agricultural field trials where this intrinsic variation is decisive for the outcome of the experiment. It is important to choose a field site with as low variation as possible, since variations in important soil properties can obscure treatment effects and make interpretation of the results difficult. Well thought-out experimental designs are important, while statistical techniques such as ANOVA, principal component analysis and geostatistics are helpful tools to use in the selection of a suitable site for a field experiment.

In the evaluation of data from a field experiment there are several factors that may more or less interfere with the results. Uncontrollable factors such as precipitation and temperature fluctuations as well as other external factors such as cropping measures and sampling procedures can sometimes hide "true" treatment effects. In these cases, it is of great importance to use correct statistical methods in the interpretation of the results. Classic methods, such as analysis of variance, complemented with a multivariate approach that has the possibility to explore general structures in the data set could prove to be very helpful.

Objectives

The overall objective of this thesis was to evaluate the effects of applying organic residues on agricultural soils. An integrated evaluation was adopted, whereby soil microbiological analyses were combined with soil chemical and physical analyses and measurements of crop yield and grain quality in order to describe and quantify positive or negative effects of the organic residues. Both classical and multivariate statistical methods were used to evaluate and interpret the results.

Specific objectives of this thesis were to:

I. Develop and evaluate a method combining near infrared reflectance (NIR) spectroscopy with principal component analysis (PCA) in order to identify an area of soil with low intrinsic spatial variation **(Paper I)**.

II. Evaluate the effects of application of organic waste on crop yield and grain quality **(Paper II)** and soil microbiological, chemical and physical properties **(Paper III)** in a four-year field experiment.

III. Evaluate whether wood ash has toxic effects on potential nitrification rate (PAO) and potential denitrification rate (PDA) and if compost mitigates these toxic effects **(Paper IV)**.

Methods and experiments

The experiments

The Organic Residues in Circulation (ORC) field experiment

ORC was established in 1998 with the purpose to evaluate the effects of different organic residues on agricultural soil. The experimental design was chosen to enable a comparison of organic residues (compost from source-separated household waste, biogas residues from source-separated household waste and anaerobically treated sewage sludge) with traditional fertilizers (liquid pig manure, cow manure and mineral fertilizer). The site chosen for the experiment was a poorly structured clay soil classified as a Eutric Cambisol (FAO, 1998). It was located close to the city of Västerås (59° 37' N, 16° 33' E) in eastern Sweden. The soil had not been fertilized with farmyard manure since 1975, and before the start of the experiment the field had been cropped mainly with cereals, oil-seed rape and legumes. Conventional soil management with annual ploughing was carried out.

The experiment (**Papers II** and **III**) was set up in a random block design with four replicates and a plot size of 90 m^2 (Fig. 2). A simple crop rotation with two crops (oats and barley) was used. Compost and biogas residues were applied in two ways: (1) as the sole fertilizer and (2) combined with mineral N. Sewage sludge, pig manure and cow manure were all combined with mineral N. All fertilizers were applied at a rate corresponding to a total of 100 kg N ha^{-1} (Table 1). The different manures were applied according to general farming practice in Sweden. Therefore, compost, sewage sludge and cow manure were spread a few days before ploughing in late autumn. Biogas residues and pig manure were spread on the seedlings immediately before stem elongation. Mineral fertilizer was applied in spring at sowing. Plant protection measures consisted of annual spraying with herbicides.

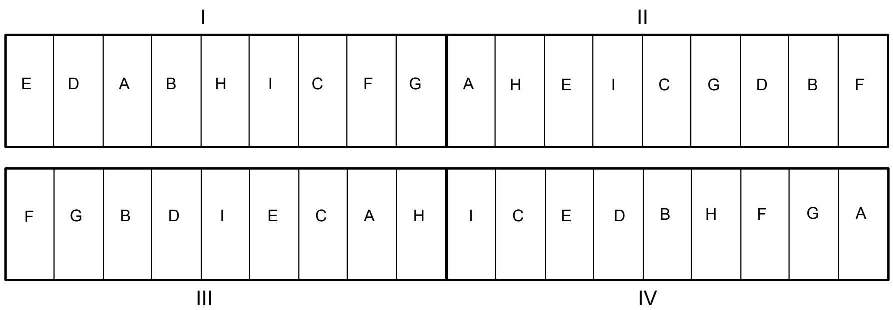

Figure 2. Design of the ORC field experiment. I, II, III and IV denote four blocks. The letters A-I indicate different fertilizers. A: compost + N, B: biogas residues + N, C: sewage sludge + N, D: pig manure + N, E: cow manure + N, F: NPS, G: control, H: biogas residues, I: compost.

Table 1. *The different treatments in the ORC-experiment. All treatments correspond to a total of 100 kg N ha^{-1} yr^{-1}*

Treatment	Residue/Fertilizer	Applied N in organic fertilizer (kg ha^{-1} yr^{-1})	Applied N in mineral fertilizer (kg ha^{-1} yr^{-1})
A	Compost + N	50	50
B	Biogas residue + N	50	50
C	Sewage sludge + N	50	50
D	Pig manure + N	50	50
E	Cow manure + N	50	50
F	NPS	0	100
G	Unfertilized	0	0
H	Compost	100	0
I	Biogas residues	100	0

The amounts of dry matter, total C, plant nutrients and heavy metals applied each year through the different treatments are presented in Table 2.

Table 2. *Mean values of annual* application *rates (n=5) of dry matter, total C, plant nutrients and heavy metals in the ORC experiment. For abbreviations, see Table 1 and Table 3*

Kg ha^{-1} yr^{-1}	A	B	C	D	E	F	G	H	I
DM	2520	287	1335	559	2252	366	0	5040	575
Tot-C	565	103	324	203	740	0	0	1130	207
Org-N	47	20	42	14	43	0	0	94	39
Min-N	3	30	8	36	7	100	0	6	61
P-Olsen	0.5	2	0.4	12	5	15	0	1	4
P-AL	6	2	6	13	10	15	0	12	5
K-AL	20	21	1	34	68	1	0	39	42
Tot-P	11	4	37	22	17	20	0	22	7
Tot-S	7	2	12	8	10	14	0	13	4
Cu[1]	170	17	430	140	75	1	0	340	35
Zn[1]	520	57	620	630	420	9	0	1030	110
Cd[1]	1	0.1	1	0.3	0.4	0.04	0	2	0.2
Ni[1]	22	3	15	4	7	3	0	44	6
Pb[1]	58	3	20	1	4	0,2	0	120	5
Cr[1]	40	7	18	17	6	12	0	81	13

[1] Expressed as g ha^{-1} yr^{-1}

The strategy in the ORC-experiment was to apply relatively small amounts of the organic residues, so that the N supply should not exceed a plant requirement of about 100 kg ha^{-1} yr^{-1}. This strategy has three significant advantages. Firstly, it avoids excess N supply that would cause leaching to groundwater. Secondly, the input of organic material resembles a natural ecosystem where the soil microbial flora is allowed to slowly proliferate and adjust to new conditions. Thirdly and most importantly, the strategy should more clearly show the effects of the different fertilizers. Since no excess N is supplied, it should be possible to see to what extent organically-bound N is mineralised and made available to the plant. In particular, the effect of combining organic residues with mineral N in contrast to the use of only organic residues will become apparent.

Soil samples were collected annually, in late autumn four weeks after harvest. Each plot was sampled about 25 times from the topsoil with soil corers (22-25 mm diam.) to obtain 4 kg of moist soil per plot. The soil samples were put in polythene bags and transported to the laboratory the same day, where they were stored at +2°C. For microbiological analyses, the soil was dried gently at +2°C, sieved, thoroughly mixed and portioned in polythene bags. The samples were then stored at -20°C and all analyses were performed within 13 months (Stenberg et al., 1998a). For chemical analyses the soil was either frozen (-20°C) or dried (35°C) and stored until analysis.

N-profile samplings were performed three times each year: in the spring, immediately after harvest and in the late autumn. Sampling depths were 0-300, 300-600 and 600-900 mm. The soil samples were stored frozen (-20°C) until analysis.

Crop yields were determined from 36 m^2 sub-plots. From each plot, 1000 g subsamples of grain were collected for further analysis. The yield figures are given at 15% water content. Immediately before harvest, full-grown plants were sampled by cutting a total area of 0.25 m^2 per plot. From these cuttings, straw yields were determined after threshing. 1000-seed-weight was analysed by weighing 200 seeds and is given at 15% moisture content.

N content in the full-grown plant was determined at three occasions during the growing seasons: immediately before spreading of the liquid fertilizers, just before ear setting and finally after blooming.

Incubation experiment

The short-term effects of wood fly ash, as well as the hypothesis that compost can mitigate possible negative effects were studied in a short-term laboratory incubation experiment (**Paper IV**). Two microbial parameters, potential nitrification (PAO) and potential denitrification (PDA), were measured after mixing either wood ash or compost as well as a combination of wood ash and compost in 24 polyethen pots filled with soil. The pots were sown with spring wheat (*Triticum aestivum*) and placed in a climate chamber with automatic light, moisture and temperature regulation. PAO, PDA, and pH were measured after a short period (7 days) and after a longer period (90 days). The application rates of wood ash and compost were based on normal application rates for agricultural fields: 6 t ha^{-1} for wood ash and 50 t ha^{-1} for compost.

A dose response assay was performed for wood ash on PAO, PDA and pH. Application rates of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8% (w/w) of wood ash was mixed into the soil and the immediate effects on PAO, PDA and pH were measured. The wood ash application rates at which PAO and PDA were reduced by 10% (EC_{10}), 50% (EC_{50}) and zero (NOEC) compared to the control were calculated using linear regression.

The metal solubility in the different treatments was examined by preparing a second set of pots and then after 7 days leaching the soil with three pore volumes of water. The eluates were then analyzed for metal content.

Chemical and physical analyses

The chemical and physical analyses in **Papers I-IV** were all performed according to standard procedures at commercial laboratories and will therefore not be discussed in any detail here. The different analyses performed on soil, crop, fertilizers (organic residues, cow manure, pig manure, NPS), wood ash and eluate are summarized in Table 3.

Table 3. *Analyses performed on soil, compost, ash, other fertilizers and the water eluate in* ***Papers I-IV****. The asterisk (*) indicates that the analysis was performed*

Analysis	Soil	Crop	Compost	Ash	R/F[1]	Eluate
pH	*					
Tot-C (total carbon)	*			*	*	
Org-C (organic carbon)			*			
Tot-N (total nitrogen)	*	*	*	*		
Org-N (organic nitrogen)			*		*	
Min-N (mineral nitrogen)	*		*	*	*	
Tot-P (total phosphorous)	*	*	*	*	*	
Tot-S (total sulphur)	*		*	*	*	
Tot-K (total potassium)		*				
P-AL (plant available phosphorous)	*		*		*	
K-AL (plant available potassium)	*		*		*	
P-Olsen (plant available phosphorous)	*		*		*	
TEB (total exchangeable base cations)	*					
CEC (cation exhange capacity)	*					
Clay content	*					
WHC (water holding capacity)	*					
Ag (silver)	*		*	*		
Al (aluminium)	*		*	*		*
As (arsenic)	*		*	*		
Be (beryllium)	*		*	*		
Ca (calcium)	*		*	*		*
Cd (cadmium)	*	*	*	*	*	*
Co (cobalt)	*		*	*		
Cr (chromium)	*	*	*	*	*	*
Cu (copper)	*	*	*	*	*	*
Fe (iron)	*		*	*		*
K (potassium)	*		*	*		*
Mg (magnesium)	*		*	*		*
Mn (manganese)	*		*	*		*
Mo (molybdenum)	*		*	*		*
Na (sodium)	*		*	*		*
Ni (nickel)	*	*	*	*	*	
Pb (lead)	*	*	*	*	*	*
Tl (thallium)	*		*	*		
U (uranium)	*		*	*		*
V (vanadin)	*		*	*		
Zn (zinc)	*	*	*	*	*	*
B-resp (basal respiration)	*					
SIR (substrate induced respiration)	*					
µSIR (specific growth rate)	*					
PDA (potential denitrification)	*					
µPDA (specific growth rate)	*					
PAO (potential nitrification)	*					
Alk-P (alkaline phosphatase activity)	*					
N-min (nitrogen mineralization)	*					

[1] Residues or fertilizers other than compost or wood ash.

Near infrared reflectance (NIR) spectroscopy

The near infrared fraction of light is defined as wavelengths between visible and mid-infrared light (700-2500 nm). In NIR-spectroscopy, a soil sample is scanned over the entire near-infrared region by use of a monochromator. In this region, each constituent of a complex organic mixture has unique absorption properties due to stretching and bending vibrations in molecular bonds (Wetzel, 1983). NIR has been used for several years in soil science for assessment of C and N (Dalal & Henry, 1986; Morra *et al*., 1991; Reeves *et al*., 2002), metal oxides (Ben-Dor & Banin, 1995) as well as moisture, cation exchange capacity (CEC), wilting point, basal respiration and soil texture (Chang *et al*., 2001a). Contrary to other spectroscopic methods, single peaks cannot be used to quantify elements or compounds in the sample. The NIR-region is dominated by weak overtones from mid-infrared fundamentals and combinations of vibrational bands of light atoms that have strong molecular bonds, for example hydrogen bonds (Wetzel, 1983; Osborne *et al.,* 1993). Large areas of the plotted spectra derived from mineral soils have the image of a smooth curve interrupted by a few broad peaks (Fig. 3). To be able to interpret NIR-spectra, multivariate calibrations are generally applied. The NIR-technique has proven to be simple, fast, cost-effective and it has the potential to replace other more expensive soil analyses. Typically, the same spectra can replace more than one analysis.

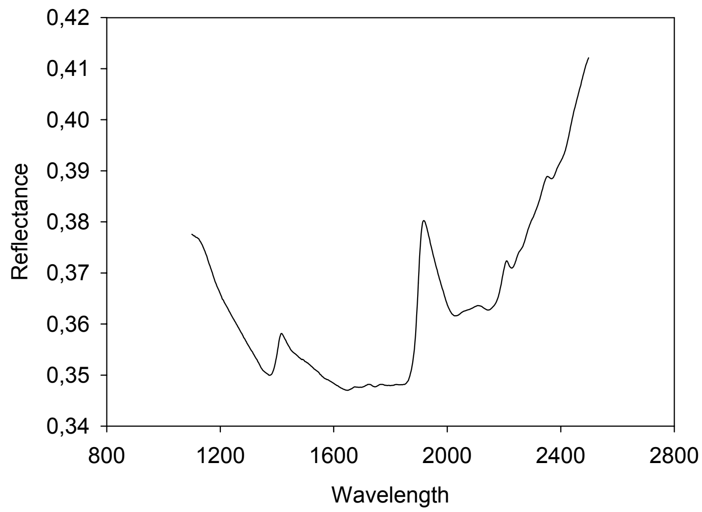

Figure 3. The NIR-spectra of one of the soil samples used in **Paper I**.

Microbiological analyses

Basal respiration (B-resp) and substrate induced respiration (SIR)

Soil respiration is defined as CO_2 evolved by soil organisms. Typically, 90% of the respiration originates from microorganisms, whereas 10% originates from soil animals. Most soil animals are eliminated during the sieving and freeze storage of the soil samples and therefore, the soil respiration may be considered as index of general soil microbial activity and is commonly regarded as the result of the overall decomposition of organic material (Anderson, 1982). CO_2-evolution, a traditional method since the early days of soil microbiology, is still considered the best index of gross metabolic activity of mixed microbial populations (Stotzky, 1997). In undisturbed soil, there is an ecological balance between the organisms and their activity. Respiration is then called basal respiration (B-resp) and is a measure of the background microbial respiration (Anderson, 1982). In this study, the B-resp was measured as CO_2-evolution after the soil had been incubated for a period of time. At the beginning of such an incubation, respiration is increased due to disturbance by sieving etc., but it declines successively to a stable level. This usually takes about one week (Martens, 1995). In the present work eight days (200 hours) was used.

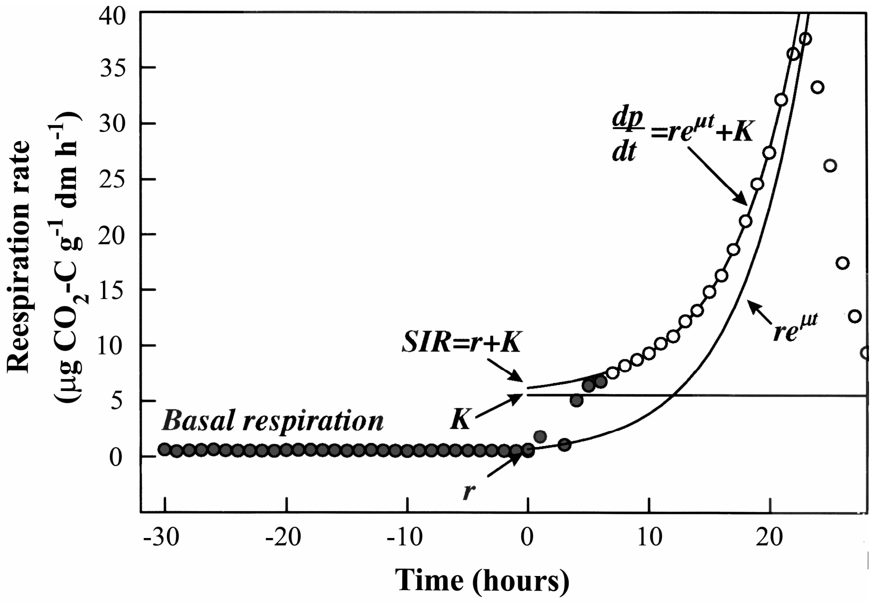

Figure 4. Illustration of the kinetic model applied for the basal respiration and substrate induced respiration. r = active (growing) and K = dormant (non-growing) microorganisms.

Microbial biomass is an important component of the soil ecosystem. Due to a lack of suitable and sufficiently standardized methods in soil microbiology, the microbial biomass pool was long neglected or estimated based on microbial counts. However, several techniques to measure microbial biomass have emerged during the last three decades. Jenkinson & Powlson (1976) introduced the chloroform fumigation-incubation (CFI) method and a modified version, the chloroform fumigation-extraction method (CFE), was described by Vance *et al.,* (1978). A physiological approach to measure microbial biomass, substrate induced respiration (SIR), was proposed by Anderson and Domsch (1978). The idea was derived from pure culture studies. Microorganisms respond to the supply of available substrate, such as glucose, with an immediate increase in respiration that was supposed to be linearly related to biomass C measured by CFI. Apart from CFI and CFE, the SIR-technique has become the most frequently used method for biomass determination in soil.

In this thesis, SIR was initiated by mixing a substrate consisting of glucose, ammonium sulphate, potassium phosphate and talcum powder into each soil sample. The parameters SIR and the specific growth rate (μSIR) were then calculated from the data by non-linear regression (Fig. 4) according to the equation suggested by Stenström *et al.* (1998). SIR was defined as the sum of activity of active (viz. growing) and dormant (viz. non-growing) microorganisms.

Potential ammonium oxidation (PAO)

Autotrophic ammonium oxidation, or nitrification, is a highly specific process carried out by only a few species of bacteria within the family *Nitrobacteriaceae.* Nitrification is a two-step process: first ammonium is oxidised to nitrite and then nitrite is further oxidised to nitrate. Their extreme specialization and a complex cell machinery makes nitrifiers sensitive to perturbations, and their activity can therefore be indicative of environmental stress factors, such as heavy metals and organic xenobiotics (Pell *et al*., 1998; Christensen *et al*., 2002). Since the process is autotrophic, it is not directly dependent on organic carbon and thus other aspects of soil quality can be emphasised.

In this thesis, nitrification was assessed as the potential ammonium oxidation (PAO) rate by a technique described by Belser & Mays (1980). The soil is incubated as an aerated slurry with excess NH_4^+ and buffered to pH 7.2. Chlorate, which inhibits the oxidation of nitrite to nitrate, is added to the soil and nitrite will therefore accumulate in the soil. During the short incubation time, no growth occurs and the product formation rate is constant. The rate of nitrite formation can therefore be determined by linear regression.

Potential denitrification activity (PDA)

Denitrification refers to the bacterial process whereby nitrous oxides, principally NO_3^- are progressively reduced in a series of enzymatic steps to the gaseous nitrogen products NO, N_2O and N_2. The nitrogen oxides act as terminal electron acceptors in the absence of oxygen. In contrast to nitrification, the denitrifying bacteria are represented within most physiological and taxonomical groups of soil bacteria. The most frequently found denitrifiers in the soil are thought to be members of the genera *Pseudomonas*, *Alcaligenes* and *Bacillus* (Zumft, 1992; Tiedje, 1988), but the list is continuously being revised as nucleic acid based methods for identification of denitrifiers become more widely applied. Most denitrifiers are heterotrophs and can mineralise easy-available carbon both under aerobic and anaerobic conditions. The denitrification process should therefore be a representative indicator for an important part of the soil microbial population.

The method used in this thesis is a modification of a method that was introduced by Smith & Tiedje (1979) and has been described and further developed by Pell (1993) and Pell *et al.* (1996). In this method, acetylene blocks the last step in the denitrification process, which results in accumulation of dinitrogen oxide in the anaerobic incubation vessel. The data is then fitted to a two-parameter non-linear model (Stenström *et al.,* 1991) (Fig. 5). The parameters describe initial production rate (PDA) and specific growth rate (μPDA).

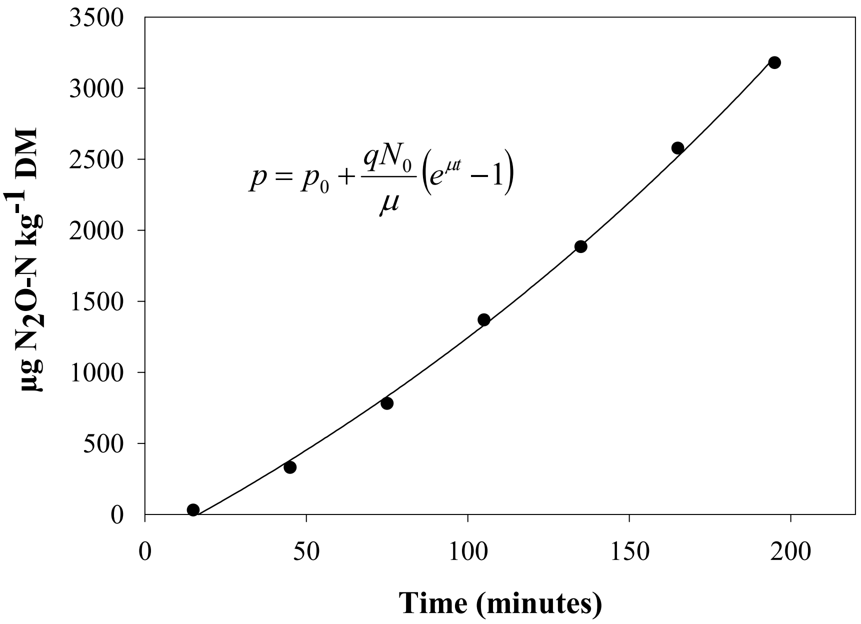

Figure 5. Illustration of the kinetic model applied for the potential denitrification activity.

Nitrogen mineralization (N-min)

The conversion of organic N to NH_4^+, a process known as N mineralization, is mediated by enzymes produced by most soil microbes and soil animals. The process can be assessed by incubation under either aerobic or anaerobic conditions to obtain an index of plant available N. Simultaneously with mineralization, NH_4^+ is incorporated partly into amino acids and nucleic acids (N immobilization), so that incubation methods yield the net production of NH_4^+. In this work, an anaerobic incubation technique with waterlogged soil was used (Waring & Bremner, 1964). The advantage with the anaerobic method is that it prevents nitrification, and thus only NH_4^+ has to be measured. Since nitrification does not interfere, incubations can be standardized at higher temperatures, and as a consequence, a shorter incubation time can be used. The rate of NH_4^+ formation was determined as the difference in the amount of product at the start and at the end of a 10-day incubation period.

Alkaline phosphatase activity (Alk-P)

P mineralization is an enzymatic process. As a group, the specific enzymes involved, called phosphatases, catalyze a variety of reactions that release phosphate to the soil solution from organic P ester compounds. Phosphatases are induced predominantly under P-limited conditions (Schinner *et al.*, 1996). There are two different groups of phosphatase enzymes: alkaline and acidic. The alkaline have their optima at pH 9 and the acidic at pH 6.5. Acidic phosphatases are generally considered to originate from both plants and soil microorganisms, while alkaline phosphatases typically are of microbial origin (Chonkar & Tarafdar, 1981).

In this thesis, alkaline phosphatase activity was assessed after a pre-incubation period of four weeks. During this pre-incubation, the activity approaches a stable level that is independent of variations induced by sample preparation (Sjökvist, 1995). A buffered (pH 9) solution of p-nitrophenol phosphate was added to the soil solution and the alkaline phosphatase activity was measured as the release of p-nitrophenol after an incubation period of two hours (Tabatabai & Bremner, 1969).

Statistical methods

Principal component analysis (PCA)

Multivariate analysis is a collective term used for a number of statistical techniques that can simultaneously account for several more or less related variables in complex environments. In PCA, a large data set of measured variables (e.g. pH, Tot-C, Tot-N) and objects (soil samples) is transformed into a new set of variables called principal components (PCs) (Esbensen, 2000). The first principle component (PC1) is the central axis that lies along the direction of maximum variance in the data set (Fig. 6). Each point (object) in the data swarm can then be projected onto this line. Therefore, the first principal component can be defined as the line that is the best fit to all the points using the principle of least-squares optimization. The second principal component (PC2) is laid orthogonally to PC1 and hence explains as much of the residual variation as possible.

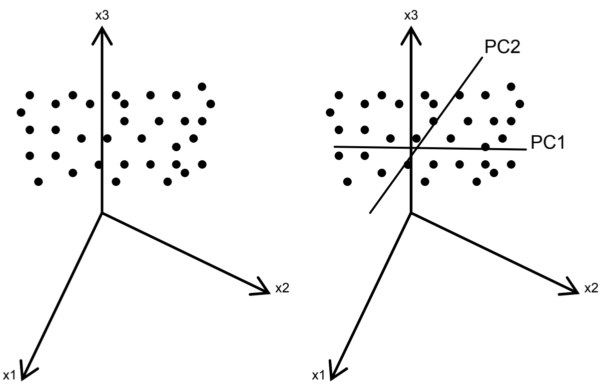

Figure 6. Schematic figure of the concept for principle component analysis (x1-x3 are different variables and the filled circles are different objects).

The score vectors (or the scores) are the orthogonal projections of each object onto each PC. Any two pair of scores can then be plotted against each other in a two-dimensional score plot to view the objects and their relative position. The loading vectors (or the loadings) are calculated as the cosine angle between a PC and a variable vector and they provide information about the relationship between the original variables and the principal components. A loading plot can therefore be used to interpret groups of objects with similar characteristics. Thus, using

PCA it is possible to create a two-dimensional window into a multidimensional space.

In **Paper I** the data set consisted of 99 objects (soil samples) and 700 variables (NIR wavelengths). PCA was used to evaluate the relation between objects differentiated by low, medium and high levels of Tot-C, clay content and pH.

In the evaluation of the ORC experiment, the relationship between the soil samples and the soil variables (chemical and microbiological analyses) were studied using PCA with clay content and block division as cofactors. This means that the effect of these two variables was removed from the PCA. Variance partitioning (Bocard *et al*., 1992; Økland & Eilertsen, 1994) was performed in order to quantify how much of the total variance was described by clay content, by block and by a combination of these two. The variance was partitioned into the following components.

A. The variance not explained by the cofactors.
B. The variance uniquely described by clay, but not by block: clay $|$ block.
C. The variance uniquely described by block, but not by clay: block $|$ clay.
D. The variance jointly described by both clay and block: clay$\cap$block.

Redundancy analysis (RDA) was used in all calculations of variance partitioning. RDA can be considered as a constrained extension of PCA that identifies trends in the scatter of data points that are maximally and linearly related to a set of constraining (explanatory) variables. Component A was calculated as the difference between the total inertia (a multivariate measure of variation in a data set) and the sum of all canonical eigenvalues in a RDA with both clay and block as explanatory variables (the latter term is noted clay$\cup$block). Component B is the sum of all canonical eigenvalues calculated using clay as the explanatory variable and block as a co-factor in a RDA. Component C was calculated the same way as component B, but with opposite roles for clay and block. Finally, component D was calculated as clay$\cup$block – B – C. All multivariate analyses used to evaluate the field experiment were performed using CANACO, ver. 4.5 (ter Braak & Smilauer, 2002).

Geostatistics

Geostatistics was originally used in the mining industry to prospect minerals (Matheron, 1963), but it has also proven to be useful in soil science to describe and understand the spatial distribution of measured soil properties. How can the continuous spatial variation of a soil property be quantitatively described? In **Paper I,** values of soil properties were estimated at points where they had not been measured. To do this we need to know how those soil properties vary spatially and to make a mathematical description of that variation. Consider a variable *Z* and suppose that *Z* has been measured at a number of sampling points in a field. The variance in these sampling points can then be calculated according to (Webster & Oliver, 2001):

$$\gamma(h) = \frac{1}{[2m(h)]}\sum_{i=1}^{m(h)}\left[Z_{(x_i)} - Z_{(x_i+h)}\right]^2 \qquad [1]$$

where $\gamma(h)$ is half the squared difference between two values, usually designated the *semivariance* for this reason, *h* is the separation distance interval, *m* is the number of data pairs within this distance interval, and $Z(x_i)$ and $Z(x_i + h)$ are the sample values at two points separated by the distance *h*.

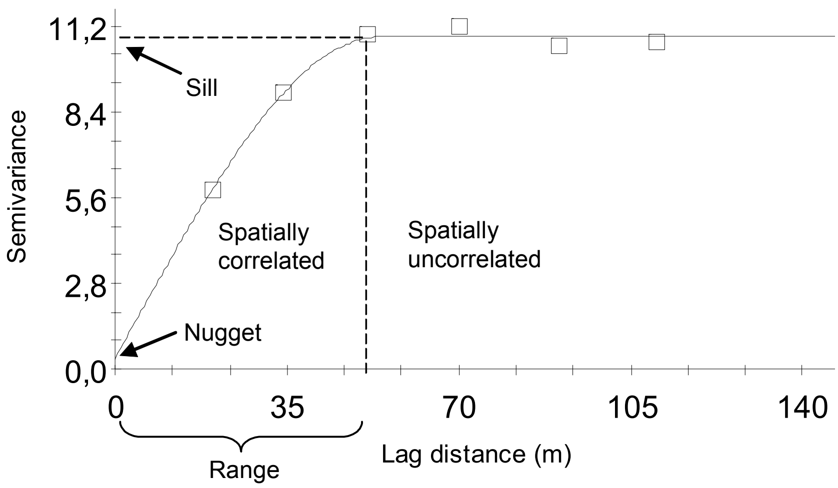

Figure 7. The semivariogram for the spatial variation of clay content in the agricultural field studied in **Paper I**.

The spatial variation in a field can then be described in a semivariogram (Fig. 7), where the model parameters nugget, range and sill can be determined. The nugget is the positive y-intercept and it corresponds to a discontinuity of the soil property, usually arising from error in the measurements or a too coarse sampling interval. The range is the separation distance where points are no longer spatially correlated and the sill is the value of the semivariance at distances greater than the range.

Analysis of variance (ANOVA)

The one-way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable. The term one-way refers to the fact that only one factor at a time varies in the experiment. For example in **Papers II, III** and **IV** the factor of interest was the type of treatment used. The treatment effect was then tested on different variables, such as crop yield, Tot-N, Tot-C, P-AL etc. ANOVA is used to test the hypothesis that several means are equal, and the technique is an extension of the two-sample *t* test. Once we have determined that differences exist among the means, 'post hoc' range tests and pairwise multiple comparisons can determine which means differ. Range tests identify homogenous subsets of means that are not different from each other. Pairwise multiple comparisons test the difference between each pair of means, and indicate significantly different group means at a specified confidence level. In **Papers III** and **IV** the one-way ANOVA procedure was performed (SPSS LEAD Technologies, Inc. 2002) to detect treatment effects among the different fertilizers. In **Paper II** and in the evaluation of crop yield for oats and barley in this thesis, the procedure Mixed (SAS Institute Inc. 1999) was used to detect significant effects of treatment, crop and year (within crop) and statistical interactions between treatment and crop and treatment and year (within crop). Tukey's significant difference test was used for multiple comparison and range tests.

General discussion

Describing spatial soil variation

In agricultural field experiments, the major interest is to detect treatment effects. A large intrinsic spatial soil variation may seriously affect the experiment and hence make it difficult to interpret the results. In statistical terms, a small spatial variation in the soil increases the probability of finding differences between treatments and hence rejecting the null hypothesis (H_0) when H_0 is actually false. This is called increasing the *power* of the test. Areas suitable for field experiments are usually selected subjectively, based on visual inspection combined with information on topography, uniformity of the soil, practices of ploughing, history of fertilization, the occurrence of pests, history of usage and the farmer's experience of growth and crop yield (Ohlsson, 1965; Hallerström, 1983). The subjective method is unsatisfactory, as it probably does not reveal enough relevant information about the spatial variation in the field. An alternative and more objective method would be to use near infrared reflectance (NIR) spectroscopy in combination with principal component analysis (PCA). The method described below was used to select the field area where the ORC Field Experiment was established. A modification of the technique is described in **Paper I**.

The selection strategy included four steps: (1) a preliminary survey to find a uniform field was carried out using the traditional procedures recommended by Ohlsson (1965) and Hallerström (1983); (2) within the uniform field a rectangle with a size of 200x160 m was selected and grid nodes of 20x20 m were marked, making a total of 99 sampling points; (3) soil samples were taken from the nodes and the soil was analyzed for NIR; (4) PCA was performed on the NIR data and, based on a map of PC1 of NIR (Fig. 8), an area large enough for a field experiment was selected that appeared uniform in the PCA-plot, with as few "peaks and valleys" as possible. The same area was then identified on a two-dimensional map and established in the field with GPS.

The first principal component (PC1) of the resulting PCA explained 61% of the total variation in the data set and another 14% was explained by PC2. By definition, PC1 always captures most of the total variation and, it is assumed, the most significant variation. In **Paper I** the semivariance of PC1 was compared with the semivariance of the soil variables Tot-C, pH and clay content. The results showed that PC1 displayed the largest range (148 m) and a large proportion of structural variance (0.999). The robust semivariogram (Fig. 9) and a nugget close to zero indicate that all variance of PC1 was well described by the lag distance. None of the other measured soil variables described the spatial variation as effectively as PC1. This means that PC1 of NIR reveals information on spatial variation that does not originate from Tot-C, clay or pH.

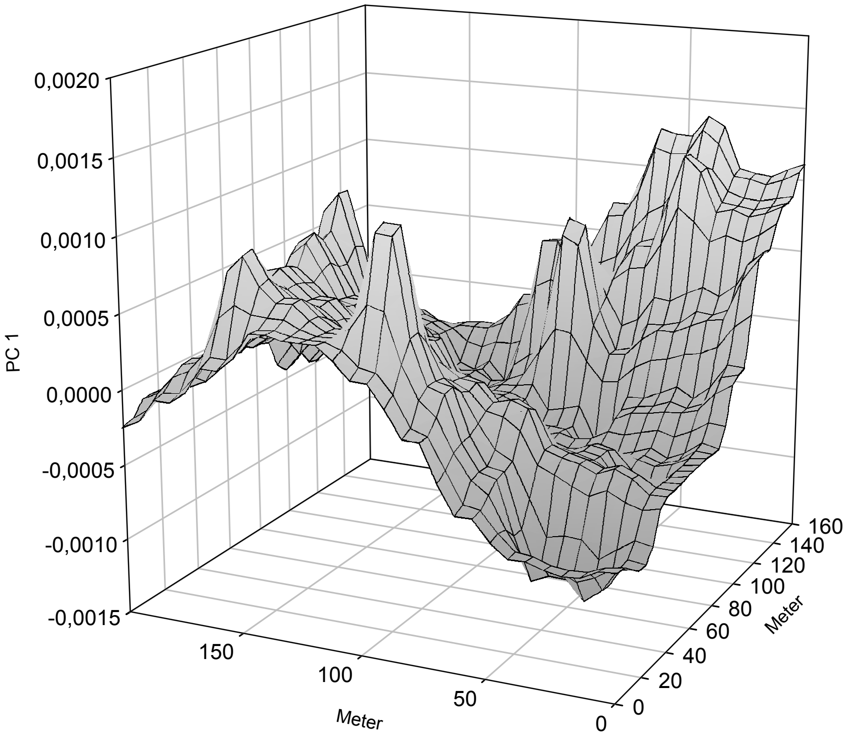

Figure 8. A three-dimensional mesh-plot of PC1 of NIR.

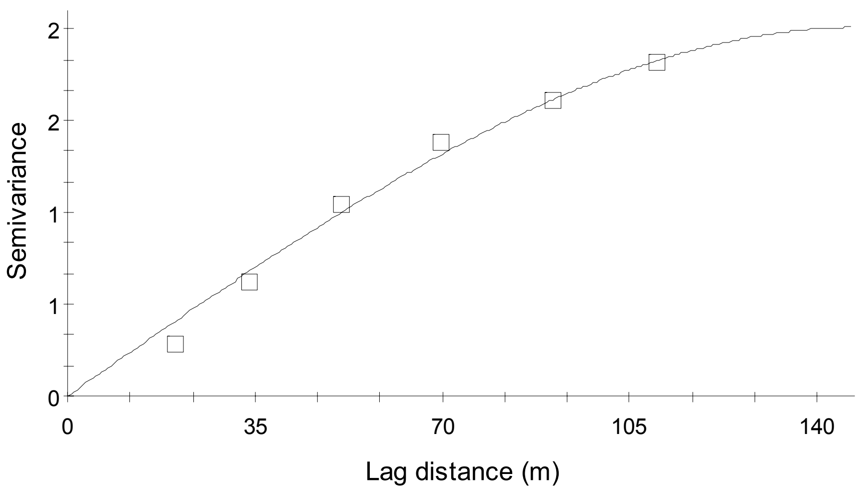

Figure 9. Semivariogram for PC1 of NIR of the soil used in **Paper I**.

It is apparent that PC1 of NIR described a large part of the variation in the soil data set, but the question is: the variation of what? The NIR spectrum holds information on many different variables and one single band in the spectrum can react to different soil properties. Therefore, it is difficult to be certain what a single wavelength reflects in terms of measurable soil variables. NIR can indicate either the actual content of a specific component in the soil or it can indicate other properties that in turn are somehow related to or affected by that component. NIR data are known to reveal information about important soil chemical (Revves *et al.,* 1999; Confalonieri *et al.,* 2001; Chang & Laird, 2002), physical (Moron & Cozzolino, 2003; Sorensen & Dalsgaard, 2005) and biological properties (Reeves *et al.,* 2000). Such variables are well known to influence soil microbial activity and plant growth. Although cheap, NIR is difficult to interpret, as it requires large reference data sets and sophisticated computer models. However, assessments of soil variation in more general terms can probably be successfully performed by the NIR-PCA strategy. By applying the PCA on the NIR data the resulting first PCs will always capture the spectral bands that express the largest variation regardless of what the bands of NIR correlate with. Consequently, PC1 ought to provide sufficient information to determine which part of the field is most uniform.

Long-term effects of organic residues

Crop yield

In the first four years of the ORC field experiment, biogas residue gave a higher yield of oats and barley than compost (**Paper II**). However, a combination of organic residue and mineral N always gave higher yields than when organic residue was used as the sole fertilizer. In Fig. 10, the crop yields for the six first years (1999-2004) of ORC are presented. Mean values for oats (the years 1999, 2001 and 2003) and barley (the years 2000, 2002 and 2004) are presented separately. Although another two years have been added, the main patterns in yield reported in **Paper II** persist. Biogas residue still gave higher yields than compost, and a combination of organic residue and mineral N was still the best choice. However, the yield of oats was no longer significantly different when biogas residue was used as the soil fertilizer compared to when the organic residues were combined with N. The overall mean crop yields for both three-year periods were very similar for oats and barely (2631 and 2637 kg ha^{-1} yr^{-1}, respectively), even though the rainy growing season in 2002 resulted in a lodged crop which significantly reduced the yield of barley in this year.

Since the application rates were relatively low, and the content of mineral N varied between the different treatments, a pronounced yield response to N could be anticipated. The advantage of biogas residue is the high content of NH_4^+-N. Bååth & Rämert (2000) reported that biogas residues gave higher yields of leek than either compost or chicken manure, while Marchain (1992) found that biogas residues increased yields in vegetable production by 6-20%. Biogas residues can also replace mineral fertilizer in cereal production (Tiwari *et al.,* 2000; Åkerhielm & Stinzing, 2005 unpublished) and Rivard *et al.* (1995) found that biogas residues increased crop yield in direct proportion to the application rate. All three organic residues combined with mineral N gave equally high yields for both oats and barley, although sewage sludge and biogas residues seemed to be slightly better. Neither of the organic residues was significantly different from cow manure, pig manure or NPS. Sewage sludge has been shown to increase the yield of canola (Banuelos *et al.,* 2004) and a leafy crop (Heras *et al.,* 2005). Mantovi *et al.* (2005) applied sewage sludge to a wheat-maize-sugar beet rotation and found that the sludge gave similar crop yields to mineral fertilizer. Warman & Termeer *et al.* (2005) reported that sewage sludge produced corn yields equivalent to that obtained using mineral fertilizer.

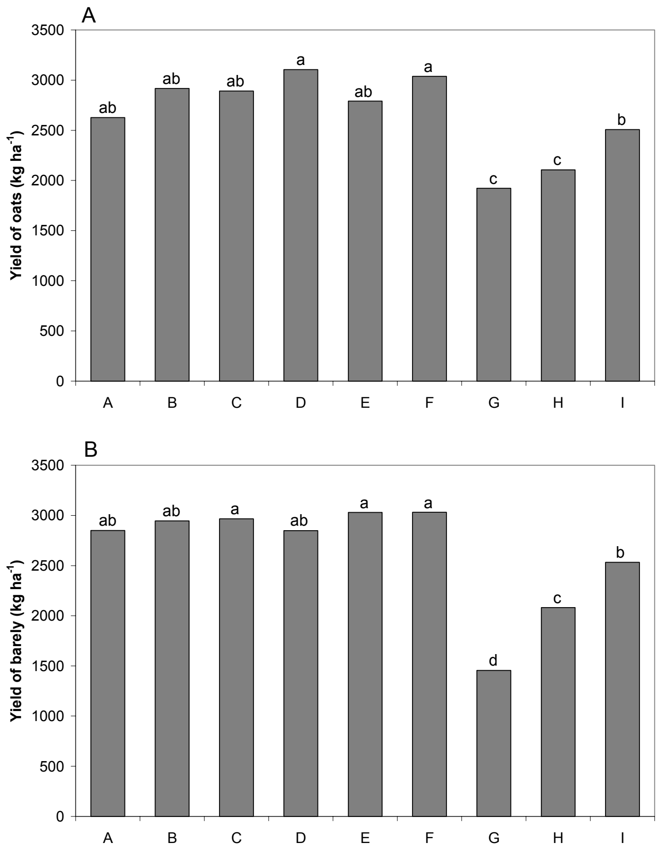

Figure 10. Mean grain yields for the years 1999-2004 for (A) oats and for (B) barley. Statistical significance (Tukey p=0.05) is indicated by different letters. For abbreviations, see Table 1.

Grain quality

The N content in the grain is an indicator of how effectively mineral N is taken up by the plant. In addition, N content in the grain is usually considered a quality measure: the higher the concentration of N, the higher the quality. In Fig. 11, the N content in the grain is presented as mean values for the years 1999-2002, that is the two different crops are treated as one. The N concentration was higher in the biogas residue fertilized crop (I) than in the compost fertilized crop (H). In addition, the concentration of N in the grain was just as large in the crop fertilized with only biogas residue as in all the other organic fertilizers and residues combined with mineral N. Apparently, the available N in biogas residues is used very effectively by the plant. The uptake of other plant nutrients or heavy metals was not significantly different between the treatments when data from the two crops were pooled and treated as one.

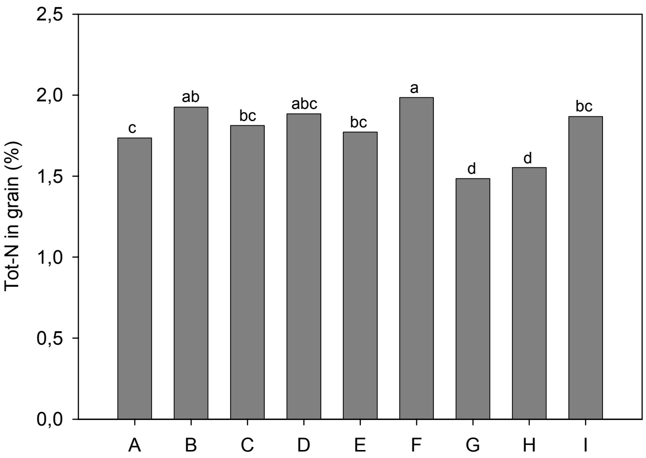

Figure 11. Mean concentrations of Tot-N (%) in the grain for the years 1999-2002. Statistical significance is indicated by different letters (Tukey's test p=0.10).

In Table 4, the concentration of Tot-N in the grain is presented for the two crops separately. For both oats and barley, fertilization with solely biogas residue generated concentrations of N equal to most other fertilizers, including NPS. Compost fertilization resulted in a lower grain N content, that was not significantly different to the control.

Table 4. *Mean Tot-N concentrations in the grain of oats (1999 and 2001) and barley (2000 and 2002). For abbreviations, see Table 1. Statistical significance between treatments is indicated by different letters (Tukey's test p=0.10)*

Treatments	Tot-N in grain (%)			
A	1.9	ab	1.6	cd
B	2.0	a	1.9	ab
C	1.9	abc	1.8	bc
D	1.9	a	1.8	ab
E	1.8	ab	1.7	bc
F	2.0	a	2.0	a
G	1.6	c	1.3	e
H	1.7	bc	1.4	de
I	1.9	a	1.8	ab

Significant differences between treatments for Cu and Ni contents in the grain were found for oats (Table 5), but not for barley. The concentration of Cu was larger in the plots fertilized with sewage sludge, pig manure and NPS than the unfertilized crop, whereas the concentration of Ni was largest in the NPS plot and smallest in the compost plot. These results do not reflect the application rates of heavy metals (Table 2), where the amount of heavy metals applied with NPS was small compared to the organic residues. For example, the compost treatment (H) supplied large amounts of several heavy metals but still the concentrations of Cu and Ni in the grain were small. Apparently, the organic material in compost immobilized the metals, so that they were not available to the plants.

Table 5. *Mean Cu and Ni concentrations in the grain of oats. For abbreviations, see Table 1. Statistical significance between treatments is indicated by different letters (Tukey's test p=0.10)*

Treatments	Cu in grain (mg^{-1} kg^{-1})		Ni in grain (mg^{-1} kg^{-1})	
A	4.6	ab	2.1	bc
B	4.8	ab	2.2	bc
C	4.8	a	2.2	bc
D	4.8	a	2.3	b
E	4.7	ab	2.3	ab
F	4.9	a	2.9	a
G	4.0	b	2.1	bc
H	4.1	ab	1.7	c
I	4.7	ab	2.1	bc

Soil chemical and microbiological properties

Since no statistical interaction was found between treatment and year, and since individual values differed greatly between the years, the chemical and microbiological soil properties were analyzed as mean values for a four-year period (**Paper III**). Although the organic residues were applied each year, no cumulative effect was found for any of the soil properties in any treatment. That is, no year-by-year increases in the element contents in the soil were observed.

Soil chemical properties

The ANOVA-procedure performed in **Paper III** showed that of all the soil chemical properties analyzed, only three of them showed significant differences between the treatments: pH, K-AL and P-AL. Compost raised the pH in the soil which has also been reported previously by Jakobsen (1995), Leifeld (2002) and Lee *et al.* (2004). On the other hand, Hartl *et al.* (2003) and Bartl *et al.* (2002) observed no increase in pH after application of compost which was most likely because the soil was more alkaline than that studied in this thesis. Compost and sewage sludge also increased the amount of available P (P-AL) in the soil and all three organic residues increased the amount of available K (K-AL) (**Paper III**). Several other authors have reported increases in soil P and K after application of compost (Steffens *et al.*, 1996; Boisch *et al.*, 1997; Adediran *et al.,* 2003) and sewage sludge (Siddique & Robinson, 2003; Banuelos *et al.,* 2004; Heras *et al.,* 2005; Mantovi *et al.,* 2005; Warman & Termeer, 2005). The increased content of plant available P in plots amended with fertilizers rich in P but poor in N, is likely to prevail as long as the application rate is based on the amount of total N. Fertilizers low in N, such as compost, will be applied in larger quantities, resulting in an accumulation of available P. None of the organic residues increased the content of heavy metals in the soil. These results are in contrast to Pinamonti (1998) and Bartl *et al.* (2002) who found increased amounts of heavy metals in the soil after application of compost. Sewage sludge application has been reported both to increase (Heras *et al.,* 2005) and not to increase (Banuelos *et al.,* 2004) the concentration of heavy metals in the soil. Dahlin et al. (1997) performed a field experiment at Brunnby Experimental farm (the same farm as was used in this thesis) where sludge had been applied at different rates for 23 years. They found that an application rate similar to the one used in this thesis significantly increased the concentrations of Cr, Cu and Zn in the soil. However, the concentrations were still smaller than the current EC limits. In the ORC field experiment, there were no significant differences between the treatments for any of heavy metals analysed. The main reason for this is that the rates of heavy metals applied were low, being less, or much less, than 1% of the original amount in the soil (Table 6).

Table 6. *Amounts of heavy metals applied in the ORC experiment. For abbreviations, see Tables 1 and 3.*

$g\ ha^{-1}\ yr^{-1}$	H	I	C	F	Soil	Doubling time (yrs) for H
Cu	340	35	90	1	51000	150
Zn	1030	115	380	9	181000	175
Cd	2	0.2	0.6	0	560	280
Ni	44	6	6	3	74000	1700
Pb	120	5	5	0.2	48000	400
Cr	81	13	13	12	40700	500

Soil microbiological properties

The majority of the microbial populations in the soil are heterotrophs and therefore dependent on the availability of organic C and energy, such as sugar, starch and cellulose. Hence, amending the soil with organic C should support a larger biomass and thereby affect respiration parameters. In **Paper III**, it was shown that substrate induced respiration (SIR) responded well to the application of biogas residues, which was expected, since it contains higher amount of easy-degradable C than compost and sewage sludge. In addition, the high NH_4^+ content resulted in increased plant growth (Fig. 1D in **Paper III**), which in turn will supply more C to the soil through litter and root exudates. The largest proportion of active microorganisms was found in plots amended with biogas residues combined with N and NPS, which were also the treatments that had the highest yields. Denitrifiers are heterotrophs and although denitrification (PDA) showed no significant treatments effects, the enzyme activity reflected in specific growth, µPDA, increased as a result of the application of biogas residues. Altogether, the C-dependent microbial parameters SIR, active microorganisms and µPDA seemed to be efficient indicators of available C in the soil. In contrast to the organic-C dependent respiration and denitrification, autotrophic nitrification bacteria are more dependent on pH and available NH_3. The nitrification process is vulnerable and is performed only by highly specialized microorganisms (Johansson *et al.,* 1999), whose activity is enhanced by NH_3 and inhibited by NO_3^-. Therefore, as expected, the NH_4^+ -rich biogas residue increased ammonium oxidation, whereas the NO_3^--rich NPS decreased it. Positive effects on soil microbial activity after amendment of organic residues have been reported in several studies. Nyberg *et al.* (2004) found that soil amendment with biogas residues under laboratory conditions increased both SIR and PAO. Compost application has been shown to increase microbial biomass and enzyme activities (Ros *et al.,* 2003; Lee *et al.,* 2004) as well as organic matter mineralization rates (Leifeld *et al.,* 2002). Zaman *et al.* (2004) found that application of sewage sludge increased microbial biomass C and N and CO_2 evolution as well as potential nitrification rate, while Barbarick *et al.* (2004) found that sewage sludge increased SIR but did not affect basal respiration. Kostov & Van Cleemput (2001) studied the effect of amending a Cu contaminated soil with sewage sludge and found that the sludge effectively promoted the recovery of soil microbial activity. Dahlin et al. (1997) evaluated the effect of 23 years of sludge application on several microbiological properties in Brunnby soil. Application rates similar to the ones used in this thesis had no significant effects on both the microbiological properties studied in this thesis. Slightly higher rates (2 tonnes ha^{-1} yr^{-1}) significantly reduced biomass C, whereas a high rate (4 tonnes ha^{-1} yr^{-1}) affected most of the microbial properties.

A multivariate approach

In the evaluation of the results of a field trial, it is important to determine whether differences in measured soil properties are due to other factors than the applied treatments. For example, gradients of clay in the field may obscure treatment effects for certain soil chemical or microbial variables that are somehow related to the clay content in the soil. The NIR-PCA method used in **Paper I**, enabled the establishment of the ORC Field Experiment in an area with little spatial variation, but there may still be small, though significant, spatial gradients (e.g. in clay content). Ideally, a randomized block design should compensate for a small degree of spatial variation in the soil, but the design is not 100% safe. Another factor that may obscure treatment effects is the block division itself. The different locations of the blocks may cause spatial variation in soil properties not related to the different treatments. In a traditional ANOVA analysis, significant differences between blocks for separate variables will appear as a block effect. In a variance partitioning carried out in a multivariate approach, all variables are considered at the same time to see how much of the total variation in the data can be explained by certain factors. A partially constrained ordination with variance partitioning of the ORC data set revealed that clay alone explained 4.7% of the variation in soil chemical and microbiological variables and block alone explained 9.7% (Fig. 12). In addition, clay and block jointly explained 7.8% of the variation. Together these three factors explained 22.2% of the total variation, which is a fairly large proportion. The remaining variation (77.8%) is caused by the other measured variables, and by other unknown factors. It can be concluded that, despite the procedure (**Paper I**) used to select the site for the field trial, and despite the randomized block design, more than 22% of the variation in the data is still explained by "undesirable" factors.

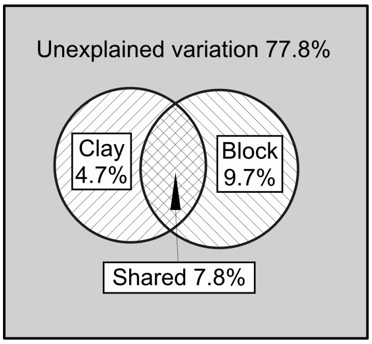

Figure 12. Partially constrained ordination with variance partitioning showing the proportion of the variance that is explained by clay alone, block alone and clay and block jointly.

Fig. 13 shows a PCA biplot of all chemical and microbiological soil variables with clay content and block as cofactors. This means that the effect of clay content and block is removed from the PCA and hence a "truer" picture of the variation appears. Arrows close to each other are positively correlated to each other, and arrows pointing in opposite directions are negatively correlated. The longer the arrow, the larger is its impact on the variance. Arrows that follow the x axis are described by PC1 and arrows that follow the y axis are described by PC2.

The most obvious pattern in the PCA is that the scores appear in four groups defined by the years 1999, 2000, 2001 and 2002. All scores denoted 99 (1999) appear in the upper right corner, the 00's appear in the upper left, the 01's in the lower left and the 02's in the lower right corner. This supports the results from the ANOVA analysis that there is a large variation in the data between the different years. Obviously, the year of the soil sampling had a larger influence on the measured soil property than the different treatments. There are no distinct groups of soil variables in the PCA-plot, but a few clusters can be detected. In the upper right corner SIR, Dormant (dormant microorganisms) and Org-C appear in the same group. This makes sense, since the respiring bacteria are heterotrophs and therefore dependent on organic C in the soil. Dormant are likely to appear close to SIR since 90% of SIR in this study was comprised of dormant microorganisms. The fact that B-resp (basal respiration) is located far from SIR suggests that B-resp responds differently to the treatments than SIR. Most heavy metals as well as Tot-S, Tot-P, Tot-N, P-AL, P-Olsen and PAO appear in the lower right corner, whereas Active (active microorganisms) and NH4-N are located in the upper part of the plot. These results can be compared to Stenberg *et al.* (1998) where PCA was used to describe the variation of certain soil variables at different scales as well as detecting functional structures in the soil. Stenberg *et al* found that certain soil variables appeared in specific groups in the PCA-plot. For example, C-related and pH-related variables were grouped in different clusters.

In addition to the clusters of variables in the ORC-data set, there may also be a weak temporal gradient in the PC2. That is, earlier years appear in the upper part of the plot, and the later years appear in the lower part. This may suggest that there is a certain accumulation effect in the soil properties after all. However, more years are needed to establish if this is the case.

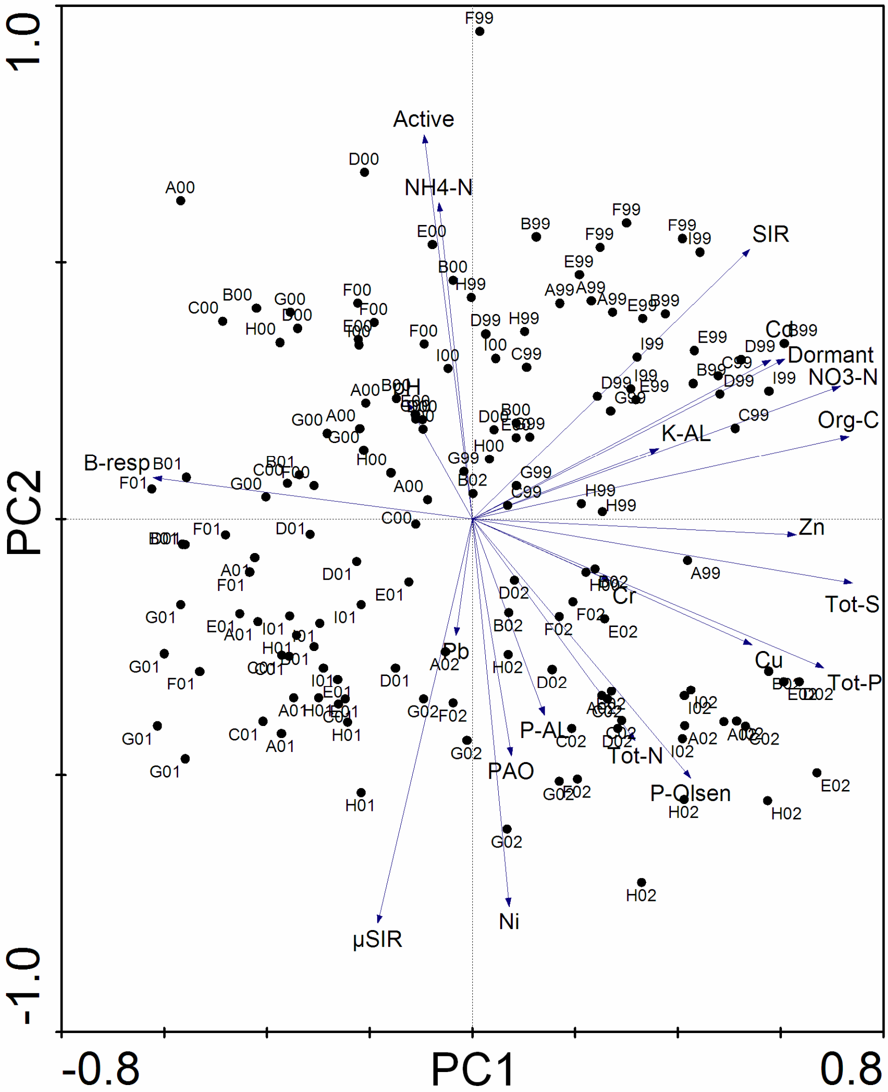

Figure 13. A PCA-biplot of the chemical and microbiological soil variables measured in the field experiment. Explained variation was 21.5% for PC1 and 17.3% for PC2. The letter and number denotes treated plots and year (e.g. A00 correspond to treatment A in 2000). For other abbreviations see Table 1.

Another pattern that appears in the PCA-plot is that some of the treatments tend to form groups within each year. For example, all G’s are more or less located in the lower left and the H’s are located in the upper part of each year-cluster. It appears that certain treatment effects can be detected within each year. A future strategy would be to run a PCA for each year and then compare the location of each treatment.

Land application of organic residues

Compared to biogas residues and sewage sludge, the mineral N content in compost is too small to meet the N requirements of agricultural plants. In addition, the mineralization rate is rather slow and only after several years of application will the organic N in compost be mineralized to the point where N becomes available to the plants. This means that it may be profitable to use compost as a fertilizer on a long-term basis. However, farmers today can seldom afford to choose long-term thinking before short-term profit. Increased application rates may of course improve the fertilizing effect, but farmers who purchase organic residues as fertilizer are interested not in maximizing the amount of material that can be applied to their fields, but rather in maximizing the net return on their investment. The soil studied in this thesis contains a relatively large amount of P and compost cannot therefore be justified as a P-fertilizer. Compost is probably best seen as a soil conditioner instead of a fertilizer and positive effects on soil structure after compost application have been demonstrated in the literature (Jakobsen, 1995). However, as long as the compost is mixed with urban park- and garden waste, there is a potential risk for accumulation of heavy metals in soils fertilized with compost. This must be taken into consideration when compost is used on agricultural soils. An alternative choice may be to use compost on urban soils, where a mulching agent is often needed and where no food crops are grown.

Biogas residues contain larger concentrations of NH_4^+ and easily degradable C, and are therefore more efficient than compost in supplying plant-available N and promoting biological activity. This, combined with the fact that biogas residues from source-separated household waste contain most other plant nutrients and very low amounts of heavy metals and other toxic compounds, suggests that biogas residues actually can and should be used as a fertilizer on agricultural soils. However, due to the relatively low content of P, biogas residues may need to be complemented with superphosphate in order to avoid P-deficits in soil. The biogas residue used in the ORC-experiment is somewhat unique, since it originates from source-separated food waste from restaurants in Stockholm. This type of residue seldom contains contaminants. Biogas residues from other sources may contain pollutants. However, a new certification system (SPCR 120) has been implemented in Sweden in order to prevent contamination of the residues.

Sewage sludge supplies the soil with sufficient K and P, but the mineral N content does not match that found in biogas residues. Sewage sludge may contain contaminants such as heavy metals and organic pollutants, although the concentrations of these contaminants are decreasing in Swedish sludge. Although several studies have reported no negative effects on soil properties after sludge application (Navas *et al.,* 1998; Johansson *et al.,* 1999; Petersen *et al.,* 2003), some studies gave contrasting results (Dahlin *et al.,* 1997; Mantovi *et al.,* 2005). However, in this thesis, no negative effects of using sewage sludge on agricultural soil were found.

Since no artificial fertilizer was used in treatments H and I (solely compost and biogas residues) results from these treatments are relevant for organic farming systems. It is clear that after a few years of application, biogas residues compare well with mineral fertilizers in terms of harvest yield. The practical on-farm handling of compost, biogas residues and sewage sludge should not pose any problem, since spreading of these residues can normally be performed with existing machinery (Ericsson, 2005 pers. commun.). The risk of NH_3 emissions following land application of the residues can be reduced by the use of shallow injection techniques (Rhode, 2004).

Selection method and statistical evaluation

A great deal of effort was put into the establishment of the ORC field experiment. NIR-measurements, PCA and geostatistics were used to select a suitable site and the experiment was designed as a block experiment with four replicates. However, despite the use of such advanced methods, more than 22% of the variation in the data was still explained by factors unrelated to treatment effects. This emphasizes how important it is to use reliable procedures to select an area with the minimum possible spatial variation. Often, this initial step is neglected when field experiments are established. It seems necessary to allocate a substantial part of the total budget for the experiment towards this initial phase of the study, considering that it may be decisive for the final outcome. Precise accuracy in the laboratory analyses may prove to be of minor importance compared to the impact of the spatial variation in the field.

Multivariate statistical methods are useful tools to evaluate data from block-designed experiments. PCA and RDA of the ORC experimental data revealed structures that could not be seen by descriptive statistics or one-way ANOVA. Obviously, interesting results may be missed if only classical statistical methods are employed.

Effects of wood ash and compost on PAO and PDA

High concentrations of heavy metals are known to be toxic to microorganisms and therefore, soil microbial responses should be considered when soil is amended with ash. The concentration of metals varies greatly in different wood ashes and authors have reported both smaller (Huang et al., 1992; Perkiömäki & Fritze., 2002) and larger (Etiegni et al., 1991a) amounts than those present in the wood ash used in this thesis. However, the heavy metal concentrations exceeded the national limits set up by the Swedish Board of Forestry (Meddelande 2001:2) for the metals As, Co, Cu, Mo, Pb and Zn. The effect of wood ash on microbial activity has mainly been studied in boreal forests (Perkiömäki & Fritze, 2002; Fritze et al., 2000; Zimmermann & Frey, 2002; Bååth et al., 1995) and similar studies have not been carried out to the same extent on agricultural soil.

Although the total metal load to the soil is important, research has shown that metal solubility and availability is also important (Basta *et al.,* 2005). Soil reactions such as sorption, precipitation and metal speciation play critical roles in determining metal solubility and bioavailability. The metal dose experienced by the microorganisms in the soil (i.e. the bioavailable fraction) is not always directly proportional to the total content of contaminants in the soil (Alexander, 1995). Amending soil with organic material has been shown to limit metal bioavailability (Basta *et al.,* 2005) and the use of organic residues for land restoration is a practice that has been successfully carried out for at least 25 years (Sopper, 1993; Haering *et al.,* 2000). Sewage sludge has been considered for land remediation (Bleichschmidt *et al.,* 1999; Delschen, 1999; Zier *et al.,* 1999) and compost has been shown to reduce the phyto- and bioavailability of several heavy metals (Brown *et al.,* 2004; Li *et al.,* 2000; Bolan *et al.,* 2003).

In **Paper IV**, an incubation experiment was carried out with the aim to test whether application of wood ash has any toxic effects on soil microbial activity and, if this is the case, whether application of compost could mitigate these toxic effects. The effect on potential ammonium oxidation (PAO) and potential denitrification activity (PDA) was assessed immediately after application and after a moderately long-term incubation of (1) wood ash, (2) compost and (3) a combination of wood ash and compost. In addition, the solubility of some specific metals was examined in soil leaching tests.

Liming effect

The results showed that all treatments (wood ash, compost and a mixture of wood ash and compost) increased the pH compared to the control after 7 days of incubation. However, the pH values in these three treatments were not significantly different from one another. Application of wood ash has generally been observed to increase pH in the soil (Etiegni *et al.,* 1991b; Bååth *et al.,* 1995; Zimmermann & Frey, 2002) and increases in pH after compost application were reported by Jakobsen (1995), Leifeld (2002) and Lee et al. (2004). After 90 days, no effect of wood ash on pH was observed and the pH was no longer different from the control. This is in agreement with Clapham & Zibilske (1992) and Muse & Mitchell (1995) who suggested that ash increases the pH in the soil, but only for a short period of time.

Potential nitrification rate

The potential nitrification rate (PAO) was increased compared to the control after 7 days of incubation of wood ash and this effect was not influenced by the addition of compost to the soil. However, after 90 days, the mixture of wood ash and compost resulted in higher PAO than all other treatments.

In contrast to the incubation experiment, the dose-response test showed that PAO decreased immediately after application of wood ash (Fig. 14). This decrease could be explained by an initial toxic effect caused by heavy metals in the ash. Inhibition of nitrification due to heavy metal exposure has been reported in several studies. For example, Cela & Sumner (2002) found that Cu and Zn impaired nitrification and Stuczynski *et al.* (2003) found that Zn had an inhibitory effect on nitrification as well as several other enzymatic activities.

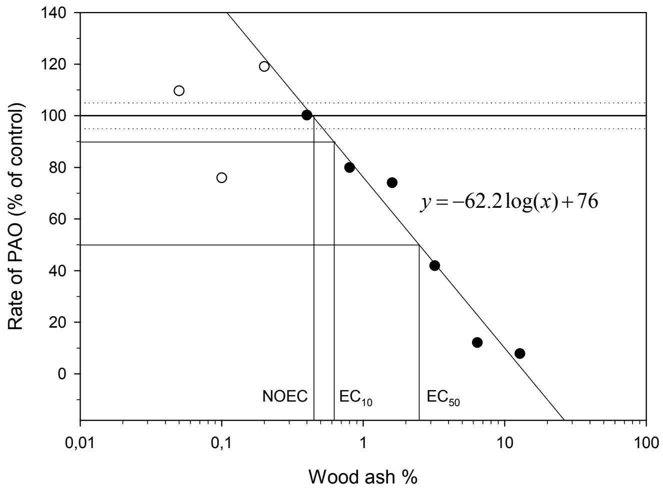

Figure 14. Changes in soil PAO after application of different rates of wood ash. Filled symbols represent data included in the linear regression.

The difference between the incubation experiment and the dose-response test suggests that the initial toxic effect of wood ash becomes less apparent after 7 days. This makes sense, since the 7-day period may allow the bacteria to grow and adapt to their new environment. The autotrophic nitrification process is sensitive to changes in pH and ash particles may form so called 'hot-spots' with higher pH in the soil. Populations of nitrifying bacteria dwelling in these sites may respond to the increased pH by enzyme production and growth and consequently increase the PAO. After 90 days, wood ash combined with compost generated the highest PAO, which was probably due to mineralization of organically-bound N in the compost.

Potential denitrification activity

In contrast to PAO, the potential denitrification activity (PDA) was reduced compared to the control after amendment with wood ash after both 7 and 90 days of incubation. In addition, the dose-response test showed decreased rates of PDA after application of wood ash (Fig. 15). Apparently, wood ash possesses some toxic properties that inhibit denitrifying bacteria. The ash contained relatively high concentrations of the metals Cd, Cr, Cu, Ni, Pb and Zn and negative effects on denitrification by various heavy metals have been shown by several authors (Bardgett *et al.,* 1994; Gumealius *et al.*, 1996; Sakadevan *et al.,* 1999; Holtan-Hartwig *et al.,* 2002). Adding compost mitigated the negative effects of the ash both in the short- and long-term, and there may be several explanations for this. The compost could have reduced the bioavailability of some heavy metals, in agreement with Brown *et al.* (2003) and Brown *et al.* (2004) who showed that compost reduced the bioavailability of Pb, Zn and Cd. It is also possible that the organic C supplied with the compost promoted the activity of heterotrophic denitrification bacteria. Compost could also promote the activity of aerobic microorganisms whose respiration will eventually result in a more anaerobic environment.

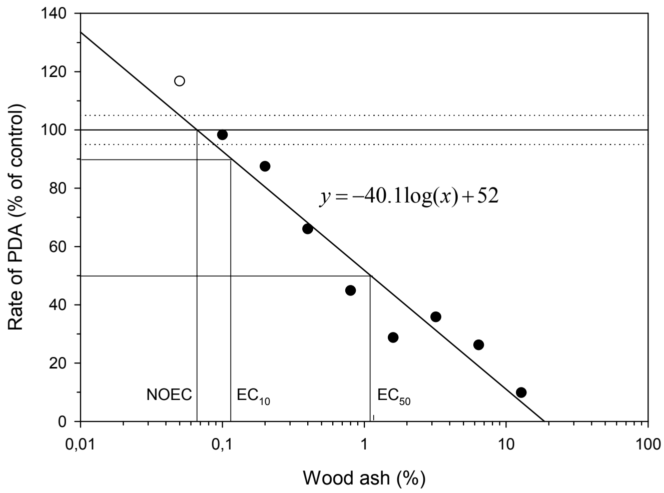

Figure 15. Changes in soil PDA after application of different rates of wood ash. Filled symbols represent data included in the linear regression.

When soil is amended with a medium such as wood ash, the specific environment for which the denitrifiers are adapted is altered and a reduction in activity is therefore to be expected. However, the fact that the inhibitory effect remained after 90 days indicates that the negative effects were severe and that adaptation of microorganisms to the new environment had yet not occurred after a substantial amount of time. Apparently, application of wood ash had a more profound influence on PDA than PAO.

Solubility of metals

The solubility of different metals varied in the different treatments, but most heavy metals leached more extensively from the compost treatment compared to the wood ash treatment. Several factors, such as humus content and colloidal properties, in combination with the characteristics of the amended material, determine the solubility of a specific metal. Compost may have promoted microbial activity, which in turn generated 'hot spots' of lower pH, which will increase the solubility of most heavy metals. In addition, metals bound to organic material were probably leached with dissolved organic matter.

Conclusions

The main conclusion of this thesis is that application of compost, biogas residues and sewage sludge has several positive effects on crop yield, grain quality and soil chemical and microbiological properties. The advantage of organic residue application instead of mineral fertilizer is the ability of the organic residue to serve as a slow-release fertilizer, which will provide nutrients not only for the first year but also for the following years. If crop growth is intense and nutrient requirements are high it may be necessary to combine the organic residue with plant-available nutrients, at least for the first few years.

Important findings are:

- Soil microbiological properties revealed treatment effects more sensitively than soil chemical properties.
- Application of compost, biogas residues and sewage sludge had no negative effects on any of the chemical or microbiological properties analysed.
- Compost should mainly be used as a soil conditioner and supplier of organic matter, since the content of mineral N is too small to serve as a fast source of plant nutrients. In addition, compost has the potential to be effective in bioremediation programmes, since it has the ability to reduce the toxic effects of heavy metals present in wood ash.
- Biogas residue contains sufficient amounts of mineral N to serve as an organic fertilizer for agricultural crops. It promotes biological activity in the soil and it increased both crop yield and grain quality.
- Sewage sludge in combination with mineral N increased crop yield and grain quality. It contains large amounts of P and can therefore be viewed as a P-fertiliser.
- Wood ash had a toxic effect on potential denitrification activity both on a short- and moderately long-term basis. Compost mitigated this toxic effect.
- It is important to thoroughly evaluate spatial variation in the soil when establishing a field experiment.
- Multivariate statistical methods effectively complement classical statistical methods since they provide an excellent overview of general trends in the data.

A field experiment like ORC becomes more valuable with time. Long-term experiments of this kind are rare and costly, but this expense must be afforded since they can give answers to the key challenge of how to utilize organic waste with minimal effect on the environment.

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