The effects of 55 years of different inorganic fertiliser regimes on soil properties and microbial community composition

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ABSTRACT

Agricultural fertilisation increases crop yields but can cause environmental damage, thus reductions in inorganic fertiliser application have been advocated. Farmer usage of phosphate rock has declined over the last decade, which may lead to soil nutrient depletion that undermines future crop production. We investigated the long-term (55 years) effects of eight different inorganic fertiliser regimes at four sites: no phosphorous and potassium (PK) fertilisation or annual replacement of harvested PK, combined with 0, 50, 100, or 150 kg nitrogen (N) ha^{-1} yr^{-1} on a range of soil properties and microbial community composition. We also investigated whether differences in microbial community composition under different fertiliser regimes arose from differences in underlying soil properties, changes in soil properties resulting indirectly from fertilisation, or directly from fertilisation. Reduced fertiliser application significantly reduced topsoil organic carbon and N, as well as plant-available P. This significantly reduced sugar beet yields but had less impact on winter wheat. The different fertiliser regimes had no significant effect on microbial community composition. Differences in soil properties as a result of fertilisation were less than differences between sites, and differences in microbial community composition were mainly explained by site. The results show that long-term inorganic fertiliser practices have little impact on microbial community composition, and lend support to research showing that microbial community composition is more influenced by organic matter inputs and underlying soil properties.

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1. Introduction

Agricultural intensification exerts strong influence on soil properties and microbial community composition (Mäder et al., 2002; Birkhofer et al., 2008). Repeated application of inorganic fertilisers decreases soil pH (Fox and Hoffman, 1981; Liu et al., 2012), which in turn can reduce nutrient availability and microbial biomass (Bardgett, 2005). Nitrogen (N) fertilisation can alter microbial community composition by increasing bacterial biomass relative to fungal biomass (de Vries et al., 2006), and can also increase soil organic carbon (SOC) by enhancing plant biomass production (Alvarez, 2005). In light of environmental problems associated with excessive fertiliser use (Foley et al., 2011), reductions in inorganic fertiliser application have been advocated (Mäder et al., 2002). Since 2008, many farmers have reduced their use of phosphorous (P) fertilisers, which coincided with an on-

going increase in the global price of phosphate rock (Cordell and White, 2011). Reductions in phosphate fertiliser application have also been documented on organic farms, and are associated with a depletion of plant-available P stocks that may undermine future crop production (Gosling and Shepherd, 2005). Long-term changes in fertiliser usage may therefore have significant effects on soil properties and microbial community composition.

Microbial community composition is strongly affected by soil chemical properties and soil organic matter content (Frostegård and Bååth, 1996; Wakelin et al., 2008; Rousk et al., 2010). However, it is unclear whether observed differences in microbial community composition under different fertiliser regimes are more a result of differences in underlying soil properties, changes in soil properties as a result of fertilisation practices (an indirect result caused, for example, by changes in soil pH or plant community composition), or a direct result of fertilisation *per se* (nutrient enrichment or depletion). Jangid et al. (2008) found that fertilisation had a significant effect on microbial community composition, but could not discern if this was due to changes in soil properties (e.g. soil pH, organic carbon and nitrogen) or a direct result of

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fertilisation. More recently, land-use history was found to have a stronger effect on microbial community composition than soil properties (Jangid et al., 2011), suggesting greater importance of human inputs (fertilisation, ploughing and vegetation cover) and their legacy. Identifying the processes that most affect soil properties and microbial community composition is important for developing sustainable agriculture (Bending et al., 2004; Alvarez, 2005).

In this study we analysed soils from four long-term fertility experiments in southern Sweden. These experiments have all received the same range of nitrogen-phosphorous-potassium (NPK) fertiliser treatments and undergone the same crop rotations since 1957. We investigated the long-term effects of these different NPK fertiliser regimes on soil properties, nutrient depletion, microbial community composition and crop yields. We also examined what the relative effects were of underlying soil properties, fertilisation, and fertiliser induced changes in soil properties on microbial community composition. We hypothesised that: 1) SOC, total nitrogen (TN) and plant-available P would all be greatest with the highest levels of NPK application and lowest in the absence of fertiliser. 2) Microbial biomass would be greatest with the highest levels of NPK application and have a lower fungal:-bacterial ratio. 3) The greatest differences in microbial community composition would be as a result of fertiliser induced changes in soil properties.

2. Materials and methods

2.1. Sampling sites

We analysed soils from four long-term fertility experiment sites in southern Sweden (Table 1) (Carlgren and Mattsson, 2001). The plots at these sites have undergone the same four year crop rotation since 1957: spring barley (*Hordeum vulgare* L.) − white mustard (*Sinapsis alba* L.) or spring oilseed rape (*Brassica napus* L.) − winter wheat (*Triticum aestivum* L.) − sugar beet (*Beta vulgaris* L.); and the same fertiliser regimes. Due to severe infections by club-root (*Plasmodiophora brassicae*) oilseed rape was replaced by oats (*Avena sativa* L.) at two sites (Orup and Ekebo). Detailed soil classifications and site land-use histories are provided by Kirchmann and Eriksson (1993) and Kirchmann et al. (1999). In 2010 the trial at Örja was moved to Borgeby due to construction work: the top 20 cm of soil was transferred.

The experiment at each site was established using a full-factorial design: four sub-plots nested within each of two main plots, giving a total of eight treatments. For the two main plots, one has received no PK fertiliser since 1957 (No PK), the other has received annual replacement of PK removed during crop harvest (Replacement PK). For the sub-plots (nested within the PK treatments) there were four N fertiliser treatments; these received an average of 0, 50, 100 and 150 kg N ha^{-1} yr^{-1} over the four year rotation. Soil was collected (15 cm depth) from the eight treatments at each site using a 3 cm diameter soil corer. Three samples were collected from each treatment at each site, and were bulked to provide a representative sample of each treatment at each site. Soils were sieved to 2.5 mm

and stored at −20 °C in plastic bags. Soil was collected in June 2012 and the current crop was sugar beet.

2.2. Soil properties and nutrient depletion

To calculate each soil's water holding capacity (WHC) soil samples were soaked in water for 48 h to reach field capacity, weighed, oven-dried at 100 °C and reweighed. Plant-available P was analysed using the Bray-1 extraction (Bray and Kurtz, 1945). SOC, TN and pH were analysed after crop harvest in 2011, as described by Kirchmann et al. (2013).

To assess the effects of topsoil nutrient depletion as a result of the different fertiliser regimes we analysed the yields of sugar beet (kg sugar beet × their sugar content ha^{-1}) and winter wheat (kg grain ha^{-1}, at 15% moisture content) over the last three rotations (2000 − 2011). Sugar beet develops a limited root system compared with cereal crops (Brown and Biscoe, 1985; Pietola and Alakukku, 2005) and is consequently restricted to exploiting topsoil nutrients. The more expansive root system of winter wheat allows it to explore greater soil depth. Differences in yield responses of the two crops would therefore highlight the extent and effects of topsoil nutrient depletion under the different fertiliser regimes.

2.3. Microbial biomass and community composition

Microbial biomass and community composition were analysed using phospholipid fatty acids (PLFA), following Frostegård and Bååth (1996). PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω7, 16:1ω9, i17:0, a17:0, cy17:0, 17:0, 18:1ω7 and cy19:0 were used to represent bacterial biomass; 18:2ω6 was used to represent biomass of saprotrophic fungi. Branched fatty acids are most associated with Gram-positive bacteria (e.g. i15:0, i16:0 and a17:0); cyclopropane and monounsaturated fatty acids are most associated with Gram-negative bacteria (e.g. cy17:0, cy19:0 and 16:1ω7c); PLFAs 10Me16:0, 10Me17:0 and 10Me18:0 have been used to represent actinomycetes (Aliasgharzad et al., 2010). However, it should be noted that these are only general associations, as individual PLFAs do not always strictly correspond to specific bacterial or fungal groups (Frostegård et al., 2011). PLFA nmol concentrations were converted to biomass C using the following factors: bacterial PLFAs: 363.6 nmol = 1 mg C; and fungal PLFA: 11.8 nmol = 1 mg C (Frostegård and Bååth, 1996; Klamer and Bååth, 2004).

2.4. Statistical analysis

Soil properties (SOC, TN, pH, P and WHC) were organised into a Euclidean distance matrix and subjected to two permutational multivariate analyses of variance (PERMANOVA) (Anderson, 2001). Each PERMANOVA incorporated the full-factorial design (main effects of PK and N plus there interaction), with non-significant terms removed sequentially. The number of permutations was set at 9999. The first PERMANOVA analysed soil properties against site; the second against PK and N treatments (permutations nested within site). Soil properties were plotted using nonmetric multidimensional scaling (NMDS). To investigate the effects of nutrient

Table 1
Soil properties and locations of the four Swedish long-term fertility experiments in Scania, southern Sweden. Different letters within columns indicate significant differences ($n = 8$).

Site	Soil texture	Soil classification	SOC (g kg^{-1})	TN (g kg^{-1})	Bray P (mg kg^{-1})	pH	WHC (%)	Latitude	Longitude
Ekebo	Loam	Haplic Phaeozem	23.5 ± 0.8[a]	1.6 ± 0.06[a]	57.8 ± 8.6[a]	6.1 ± 0.06[a]	50.5 ± 1.0[a]	55° 99′	12° 87′
Fjärdingslöv	Sandy loam; 10−20% clay	Haplic Phaeozem	11.5 ± 0.4[b]	1.0 ± 0.04[b]	22.8 ± 4.5[b]	6.7 ± 0.02[b]	48.3 ± 0.3[ab]	55° 40′	13° 23′
Orup	Sandy loam	Haplic Phaeozem	16.5 ± 0.3[c]	1.3 ± 0.03[c]	38.5 ± 3.9[a]	5.7 ± 0.07[c]	54.8 ± 0.7[a]	55° 82′	13° 50′
Örja	Clay loam	Eutric Cambisol	9.6 ± 0.2[d]	0.9 ± 0.03[d]	19.5 ± 3.4[b]	6.6 ± 0.03[b]	47.9 ± 2.1[b]	55° 88′	12° 87′