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Specific response of fungal and bacterial residues to one-season tillage and repeated slurry application in a permanent grassland soil

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ABSTRACT

The dynamics of fungal and bacterial residues to a one-season tillage event in combination with manure application in a grassland soil are unknown. The objectives of this study were (1) to assess the effects of one-season tillage event in two field trials on the stocks of microbial biomass, fungal biomass, microbial residues, soil organic C (SOC) and total N in comparison with permanent grassland; (2) to determine the effects of repeated manure application to restore negative tillage effects on soil microbial biomass and residues. One trial was started 2 years before sampling and the other 5 years before sampling. Mouldboard ploughing decreased the stocks of SOC, total N, microbial biomass C, and microbial residues (muramic acid and glucosamine), but increased those of the fungal biomarker ergosterol in both trials. Slurry application increased stocks of SOC and total N only in the short-term, whereas the stocks of microbial biomass C, ergosterol and microbial residues were generally increased in both trials, especially in combination with tillage. The ergosterol to microbial biomass C ratio was increased by tillage, and decreased by slurry application in both trials. The fungal C to bacterial C ratio was generally decreased by these two treatments. The metabolic quotient qCO_2 showed a significant negative linear relationship with the microbial biomass C to SOC ratio and a significant positive relationship with the soil C/N ratio. The ergosterol to microbial biomass C ratio revealed a significant positive linear relationship with the fungal C to bacterial C ratio, but a negative one with the SOC content. Our results suggest that slurry application in grassland soil may promote SOC storage without increasing the role of saprotrophic fungi in soil organic matter dynamics relative to that of bacteria.

1. Introduction

Conversion of perennial grassland into agricultural cropland typically results in the degradation of soil and water quality as well as in dramatic shifts in soil biota and the ecosystem services they provide (DuPont et al., 2010; Strickland and Rousk, 2010; Wakelin et al., 2013). Effects of permanent conversion have been the focus of most research, while information on grass-arable rotation and/or occasional ploughing is rare, amino sugar derived microbial residues in particular. Tillage of grassland soil and the replacement of perennial plant communities with annual crops are the two fundamental practices that affect the soil ecosystem. A strong decline in soil organic C (SOC), total N, and soil structure as well as a change in microbial community structure towards bacteria occurs after repeated tillage (Six et al., 2006; Quincke et al., 2007; Wortmann et al., 2008). Not only the long-term but also a single mouldboard ploughing event after 20 years of minimum tillage reduced the SOC stocks by 5.3 t ha^{-1} within five months after the tillage event (Stockfisch et al., 1999).

Repeated tillage has especially negative impacts on microbial biomass C (Quincke et al., 2007; Wortmann et al., 2008) and causes a shift in the microbial community structure from fungi to bacteria (Wakelin et al., 2013). Other investigations revealed a high resilience of the original microbial biomass and community structure to tillage-induced manipulations of the grassland vegetation (Potthoff et al., 2006). In accordance with this, Linsler et al. (2013) did not detect significant effects of a single cultivation event on the SOC stocks at 0–40 cm depth, although a significant decrease occurred at 0–10 cm depth. However, this discrepancy may be due to different site characteristics, e.g. clay content, soil pH and climate. Also, differences in sampling strategy might contribute to the presence or absence of tillage effects.

The fraction of non-living microbial residues contributes to long-term sequestration of C in soil and represents a significant SOC pool, larger than the living biomass (Strickland and Rousk,

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2010). Microbial residues can be assessed by measuring amino sugars, which are exclusively microbial products in soil (Amelung et al., 2008). The determination of fungal glucosamine and bacterial muramic acid gives an additional opportunity to estimate the contribution of these two main microbial groups to C sequestration (Joergensen and Wichern, 2008). Very few studies have examined the rate or extent of re-establishment of fungal and bacterial residues following the restoration of permanent grassland (Lauer et al., 2011), while the effects of one-season cultivation events are unknown. This leads to the hypotheses that (1) one-season tillage in grassland soil lead to a decrease in microbial residues, which responds faster than SOC but slower than the microbial biomass to tillage. Fungi are important components of terrestrial ecosystem, especially in respect to the decomposition of organic matter and C sequestration in soil (Jastrow et al., 2007; Strickland and Rousk, 2010). Fungal hyphae also contribute significantly to soil aggregation (Six et al., 2006; Pokharel et al., 2013). Most work has shown that fungal hyphae are more sensitive to tillage, fertilisation and land use intensity than bacterial polymers (Six et al., 2006; Strickland and Rousk, 2010). This leads to the hypotheses that (2) fungal residues react more sensitively to agricultural practices than bacterial residues.

The application of cattle manure repeatedly resulted in positive effects on SOC in grassland soil (Vertès et al., 2007) and in aggregate formation in no-till soils (Six et al., 2006). However, nothing is known about the timescale or mechanisms regulating the possible response of microbial residues to manure application in combination with a one-season cultivation event in a grassland soil. In arable soils, manure application reduced the occurrence of saprotrophic fungi (Scheller and Joergensen, 2008; Heinze et al., 2010a) and especially promoted the formation of bacterial residues, leading to increased SOC stocks (Joergensen et al., 2010). This results in the hypotheses that (3) manure application intensifies the negative effects of tillage on soil fungal biomass and residues.

These three hypotheses were investigated in two field trials with the objectives (i) to assess the effects of one-season tillage on the stocks of SOC, total N, microbial biomass, fungal biomass, and microbial residues in comparison with permanent grassland and (ii) to determine the effects of manure application performed to restore the negative effects of tillage on reduction of microbial biomass and residues. One trial was started 2 years before sampling and the other 5 years before sampling.

2. Materials and methods

2.1. Study site and experimental layout

The experimental site Lindhof is located north of Kiel (54°27′ N, 9°57′ E), Germany, near the Baltic Sea. The mean annual temperature in the area is 8.9 °C and precipitation is 768 mm (Linsler et al., 2013). In 1994, the arable land at the site was converted to permanent grassland. The grass was generally cut four times each year for forage production (Schmeer et al., 2009). Fertilisation was done during the year 2007 and 2009 and amounted to 100 kg ha^{-1} K, 24 kg ha^{-1} Mg and 68 kg ha^{-1} S and 45 kg ha^{-1} P in the form of rock phosphate in each treatment. The site has been managed organically since 1993.

Tillage trial 5 years before sampling: In 2005, a field experiment was initiated to determine the long-term effects of fertilisation of grassland soils with cattle slurry on N fluxes and C storage. In addition, a treatment with one-season cultivation of winter wheat was included to investigate the effects on nitrate leaching and on the use efficiency of mineralised N. After winter wheat cultivation, grassland was re-established to investigate the dynamics of soil organic C (SOC). The 4 treatments were (i) permanent grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (P5+), (ii) permanent grassland without slurry application (P5−), (iii) re-established grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (R5+) and (iv) re-established grassland without slurry application (R5−). All four treatments were arranged in a randomised block design with three replicates (block size 3 m × 18 m). The R5+ and R5− plots were ploughed in October 2005 and winter wheat (*Triticum aestivum* L. variety Bussard) was sown. After wheat harvest, the plots were ploughed again to incorporate the straw (approximately 7 t ha^{-1}) in September 2006 and a grass clover mixture was sown. The mixture contained 67% perennial ryegrass (*Lolium perenne* L.), 17% timothy-grass (*Phleum pratense* L.), 10% smooth meadow-grass (*Poa pratensis* L.) and 6% white clover (*Trifolium repens* L.). At the time of sampling, the surface of the treatments P5+ and R5+ was covered with 29 and 13% clover plants, respectively, whereas that of the treatments P5− and R5− was covered with 23 and 31% clover plants, respectively (Shimeng Chen, personal communication). The soil of this first trial contained 53% sand, 29% silt and 18% clay at 0–40 cm depth and was classified as Eutric Cambisol (FAO, 2006).

Tillage trial 2 years before sampling: the previous trial was repeated in an adjacent area about 50 m away with tillage events in October 2008 and September 2009. The four treatments were (i) permanent grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (P2+), (ii) permanent grassland without slurry application (P2−), (iii) re-established grassland with cattle slurry application (240 kg N ha^{-1} a^{-1}) (R2+) and (iv) re-established grassland without slurry application (R2−). All four treatments were arranged in a randomised block design with three replicates (block size 18 m × 3 m). The soil of this second trial contained 66% sand, 21% silt and 13% clay at 0–40 cm depth and was also classified as Eutric Cambisol (FAO, 2006).

2.2. Soil sampling and chemical analysis

All soil samples were taken in October 2010 in a grid design with four replicates from each block at 0–5, 5–10, 10–20, 20–30, 30–40 cm depth using a steel corer with 4 cm diameter. This resulted in 12 samples per treatment and depth in both tillage trials. All samples were passed through a 2 mm sieve and stored at 4 °C until the assessment of biological properties. Bulk density was calculated from core dry weight divided by volume. Flint stones (sedimentary cryptocrystalline form of the mineral quartz) were observed in both tillage trials, especially in the second trial. The average stone concentrations at 0–40 cm depth were 12 and 21% in the first (2005) and second trial (2008), respectively. Therefore, volume of soil (cm^{-3}) was calculated by subtracting total soil volume taken by volume of stones or the volume of the fraction of fragments > 2 mm (Schrumpf et al., 2011). A field moist soil sample was used to analyse pH (1:2.5 soil water ratio). Dried (24 h at 105 °C) and finely ground samples were used for chemical analyses (C and N). Total C and N were determined by gas chromatography using a Vario EL (Elementar, Hanau, Germany) analyser (Heinze et al., 2010b).

2.3. Microbial activity and biomass indices

The basal respiration of soil was measured by the incubation of 60 g soil sample for seven days at 22 °C with 40% WHC. The emitted CO_2 was trapped in 0.5 M NaOH and the excess NaOH was back titrated using 0.5 M HCl after the addition of saturated $BaCl_2$ solution. Fumigated (24 h with ethanol-free $CHCl_3$ at 25 °C) and non-fumigated 5-g samples were extracted with 20 ml of 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany) to measure microbial biomass C and N (Brookes et al., 1985; Vance