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Reduced tillage stimulated symbiotic fungi and microbial saprotrophs, but did not lead to a shift in the saprotrophic microorganism community structure

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ABSTRACT

The need for sustainable agricultural systems, which for example enhance soil organic carbon (SOC) content, has increased the interest for management with reduced tillage. In this study we used a Swedish long-term (20 yrs.) systems experiment, including reduced tillage (harrowing 10 cm) and plowing (moldboard plow 0–20 cm) combined with three levels of nitrogen (N) fertilization. With this setup we tested if (1) the arbuscular mycorrhizal fungi (AMF) concentration and (2) the fungi to bacteria (F:B) ratio would be higher under reduced tillage than under conventional tillage, and if this would be associated with higher SOC concentrations. We also tested if (3) the microbial biomass C close to the surface would be higher under reduced tillage than conventional tillage. Furthermore, since disturbance can reduce respiration and microbial growth we tested if (4) this occurred in our reduced tillage system. In addition, we tested if (5) fertilization increased the growth rate of fungi and decreased that of bacteria. We collected soil samples in July and October and found that the microbial biomass C, measured in October only, was higher close to the surface in the reduced tillage treatment and so was the microbial respiration. The fungal and bacterial growth rate, on the other hand, were not affected by tillage treatment. Fertilization did not affect the bacterial growth rate but did have a positive effect on fungal growth rate. In accordance with our expectations reduced tillage had a stimulating effect on AMF and saprotrophic fungi, and contrary to our expectation, also bacteria were positively affected by reduced tillage. In line with the unchanged F:B ratio, we found no indication that even 20 years of reduced tillage increased SOC concentrations in the long term.

1. Introduction

Conventional tillage, with intensive moldboard plowing, which was introduced with the industrial revolution, has led to decreases in soil organic carbon (SOC) content. When the SOC content reaches below 2%, the soil erodibility increases (Greenland et al., 1975), thus, SOC loss is a problem. Soil erosion and increased fuel prices are two factors which are often used to explain why less intensive soil management, such as the use of conservation tillage, has increased during the last decades (Zentner et al., 1996; Pimentel et al., 1995). Conservation tillage is an umbrella term encompassing several tillage methods, like shallow tillage and strip tillage, for which the soil disturbance is reduced by not inverting the soil or by decreasing the proportion of soil disturbed by tillage. Another management option available for farmers wanting to reduce soil disturbance is complete exclusion of tillage, so called no-till. The practice of conservation tillage involves leaving crop residues on the soil surface to a larger extent than when plowing is applied (Phillips et al., 1980). The crop residue layer on the soil surface helps to alleviate some of the erosion caused by wind and rainfall.

Conservation tillage is being used and evaluated for its potential to increase soil C sequestration (Puget and Lal, 2005; Stenberg et al., 2000). In order to increase soil C sequestration, the C input from e.g. plant litter and roots must exceed the loss through respiration, erosion, and leakage (Six et al., 2006). Modification of the microbial community has been suggested as part of the solution as it may reduce SOC loss. Both arbuscular mycorrhizal fungi (AMF) and saprotrophic fungi are known to be sensitive to soil disturbance caused by tillage (Helgason et al., 2010; Wardle 1995). Consequently, AMF often increase in

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abundance under conservation tillage (McGee et al., 1997). AMF is beneficial for soil structure as they are known to trap and stabilize soil aggregates with their extraradical hyphae. In addition they excrete glomalin-related proteins, which bind soil particles together (Borie et al., 2008; Bronick and Lal, 2005; Wright and Upadhyaya, 1996). Based on the assumption that stable aggregates protect carbon compounds (Grandy and Robertson, 2007), it has been hypothesized that a higher abundance of AMF can contribute to C sequestration (Wright et al., 2000). Furthermore, decreased soil aggregate disruption may also lead to lower microbial respiration and activity (Elliott, 1986; Miller, 2000), thus potentially influencing SOC.

Another way by which conservation tillage has been suggested to increase SOC content, and possibly C sequestration, is that saprotrophic fungi are favored over bacteria, due to the decreased soil disturbance. It has been argued that a higher proportion of saprotrophic fungi leads to a system with higher C use efficiency, since relatively more C is allocated to biomass instead of being respired in saprotrophic fungi than in bacteria (Ahl et al., 1998; Frey et al., 1999; Six et al., 2006). Also, fungal residues are often more recalcitrant than those of bacteria, which should increase SOC content (Guggenberger et al., 1999; Martin and Haider, 1979). However, the relative proportion of saprotrophic fungi to bacteria (F:B ratio) have been shown to both increase and decrease due to tillage (Bailey et al., 2002; Guggenberger et al., 1999; Mulder and Elser, 2009), and the practice of using the F:B ratio as an indicator for C sequestration potential has been criticized recently because of trait overlaps between fungi and bacteria (Rousk and Frey, 2015; Strickland and Rousk, 2010; Thiet et al., 2006). Thus, there is still no consensus on how tillage change affects the F:B ratio and if a high ratio is associated with more SOC.

As an effect of the reduced soil mixing, crop residues stratify within the soil profile under conservation tillage (Arshad et al., 1990). The crop residue distribution can affect the vertical distribution of microorganisms, and for example both Madejón et al. (2007) and Mohammadi (2011) found more microbial biomass C close to the soil surface under no-till compared to under conventional tillage. An altered vertical distribution of crop residues and associated changes in microbial communities and activities could contribute to long-term changes in SOC concentrations when converting from a conventional to a reduced tillage system.

In addition to tillage, one factor that can alter the F:B ratio, and possibly SOC concentrations, is addition of N containing fertilizers (Bardgett and McAlister, 1999; de Vries et al., 2006; Lupwayi et al., 2012). Increased N fertilization has been shown to decrease the F:B ratio (Bardgett et al., 1999), saprotrophic fungal hyphae length (Bittman et al., 2005), microbial respiration (Ramirez et al., 2010), and microbial biomass in general (Treseder, 2008). N-fertilization may, however, stimulate fungal growth while inhibiting that of bacteria (Bardgett et al., 1999; Demoling et al., 2007; Rousk and Bååth, 2007), even though bacterial biomass has, in some cases, been shown to increase with fertilization (Högberg et al., 2003). Direct fertilization effects on SOC are site and management dependent, and the SOC concentration has been known to either increase (Blevins et al., 1977; Paustian et al., 1997) or remain relatively unaffected by fertilization (Dolan et al., 2006).

Due to the many ways by which SOC and microorganisms respond to agricultural management, studies which capture some of the complexity, by including both tillage treatments and fertilization, are useful. The aim of this paper was to study the effects of conventional and reduced tillage on microorganisms and SOC under different N fertilization treatments, using a Swedish long-term cropping systems experiment. Based on the assumption that conservation tillage spares fungi, we tested if (1) the AMF concentration and (2) the F:B ratio would be higher under reduced tillage than under conventional tillage, and if this would be associated with higher SOC concentrations. We also tested if (3) the microbial biomass C close to the surface would be higher under reduced tillage than conventional tillage, as expected due to residue stratification. Since reduced tillage can reduce respiration and microbial growth rate we tested if (4) this occurred in our system. Also, we tested if (5) fertilization increased the growth rate of fungi and decreased that of bacteria.

2. Materials and methods

2.1. Site description, experimental setup and sampling

The fields used in our study were part of the Long-term Integrated cropping system for Nitrogen and Carbon research (LINC) systems experiment, at the Swedish Infrastructure for Ecosystem Science, SITES, Lönnstorp Field Research Station in Alnarp (coordinates 55.666252, 13.115851), in southern Sweden (Lönnstorp, 2015). The experiment was initiated in 1993 and was managed by the Swedish University of Agricultural Sciences (SLU) until termination in 2014. The soil is a Eutric Cambisol (FAO classification) with 3% organic material, 10–15% clay and 40–50% sand, and a soil pH ranging from 6.4 to 7.4 (Nilsson and Christensson, 2010). Mean annual temperature at the site is 7.7 °C and the mean annual precipitation is 537 mm (Swedish meteorological and hydrological institute, 2017). The LINC experiment included six fields managed with conventional tillage including moldboard plowing with soil inversion, 20–25 cm depth, (1 ha each), and six fields managed with reduced tillage, topsoil (0–10 cm) harrowing without soil inversion (3 ha each). During 1993–2000 reduced tillage fields were tilled, without soil inversion, down to a maximum of 25 cm depth for some crops. However, in 2000–2014 the tillage depth was reduced to 10 cm, with a likely maximum error of ± 2 cm. In 2012 one of the reduced tillage fields (field pair 5, see definition in Section 2.1) was tilled to a depth of 17 cm.

Each field contained two replicates of an N fertilization sequence treatment containing six plots (4 × 8 m). The N fertilization plots were arranged in a row, with no addition (N0) in the first plot followed by five plots with increasing rates of N addition (N1-5). The N fertilization for N3 corresponded to common practice fertilization in the region. The absolute application rates of fertilizer were adapted to the particular crop; for example, 300 kg N ha^{-1} was the highest rate applied for N5, for oilseed rape (*Brassica napus* L.) and winter wheat (*Triticum aestivum* L.), while the lowest rate for N5 was 210 kg N ha^{-1} for sugar beets (*Beta vulgaris* ssp. *vulgaris* var. *altissima* L.) (Nilsson and Christensson, 2010). The experiment followed a six-year crop rotation, and the species composition differed slightly between the tillage treatments (Table 1). Weeds, diseases and pests were managed according to conventional practices, for details see Nilsson and Christensson (2010).

We selected three field pairs in a spatial pairwise design (field pair 1, 4 and 5, see Nilsson and Christensson, 2010), each containing one conventional and one reduced tillage field, for sampling during summer (July) and fall (October). In 2013, oilseed rape and wheat were harvested in late summer (August 7th and 20th, respectively) and sugar

Table 1
Preceding and standing crop species.

	Field	Preceding crop	July crop	October crop
Conventional tillage	1	Oilseed rape	Winter wheat	Winter wheat (stubble)
	4	Barley	Sugar beet	Sugar beet
	5	Spring barley	Oilseed rape	Winter wheat
Reduced tillage	1	Oilseed rape	Winter wheat	Oil radish
	4	Barley/Oil radish	Sugar beet	Sugar beet
	5	Grassland	Oilseed rape	Winter wheat

Crop rotation for field pairs 1, 4 and 5 at Lönnstorp in 2013. Overview of the crops grown in the two tillage treatments during the sampling in July and October and also the preceding crop.