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Abundance, production and stabilization of microbial biomass under conventional and reduced tillage

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ABSTRACT

Soil tillage practices affect the soil microbial community in various ways, with possible consequences for nitrogen (N) losses, plant growth and soil organic carbon (C) sequestration. As microbes affect soil organic matter (SOM) dynamics largely through their activity, their impact may not be deduced from biomass measurements alone. Moreover, residual microbial tissue is thought to facilitate SOM stabilization, and to provide a long term integrated measure of effects on the microorganisms. In this study, we therefore compared the effect of reduced (RT) and conventional tillage (CT) on the biomass, growth rate and residues of the major microbial decomposer groups fungi and bacteria. Soil samples were collected at two depths (0–5 cm and 5–20 cm) from plots in an Irish winter wheat field that were exposed to either conventional or shallow non-inversion tillage for 7 growing seasons. Total soil fungal and bacterial biomasses were estimated using epifluorescence microscopy. To separate between biomass of saprophytic fungi and arbuscular mycorrhizae, samples were analyzed for ergosterol and phospholipid fatty acid (PLFA) biomarkers. Growth rates of saprophytic fungi were determined by [^{14}C]acetate-in-ergosterol incorporation, whereas bacterial growth rates were determined by the incorporation of ^{3}H-leucine in bacterial proteins. Finally, soil contents of fungal and bacterial residues were estimated by quantifying microbial derived amino sugars. Reduced tillage increased the total biomass of both bacteria and fungi in the 0–5 cm soil layer to a similar extent. Both ergosterol and PLFA analyses indicated that RT increased biomass of saprophytic fungi in the 0–5 cm soil layer. In contrast, RT increased the biomass of arbuscular mycorrhizae as well as its contribution to the total fungal biomass across the whole plough layer. Growth rates of both saprotrophic fungi and bacteria on the other hand were not affected by soil tillage, possibly indicating a decreased turnover rate of soil microbial biomass under RT. Moreover, RT did not affect the proportion of microbial residues that were derived from fungi. In summary, our results suggest that RT can promote soil C storage without increasing the role of saprophytic fungi in SOM dynamics relative to that of bacteria.

1. Introduction

The intensification of agriculture in the 20th century has caused several environmental problems (Tilman, 1999). High N fertilizer rates have increased nitrate leaching and N_2O emissions from cropping systems, giving rise to ecosystem eutrophication and adding to the accumulation of greenhouse gases in the atmosphere (Galloway et al., 2004). Moreover, intensive soil cultivation of arable land has lead to a loss of soil C, thereby contributing to anthropogenic CO_2 emissions (Smith, 2004). These issues spurred research interest in less intrusive agricultural management practices, and their potential to reverse some of modern agriculture's negative side effects.

The impact of management practices on the flow of C and N through ecosystems is largely mediated through the soil microbial community. Soil ecological studies often distinguish between the two main microbial groups of fungi and bacteria. Soil microbial communities with a relatively high fungal biomass have been associated with reduced N losses (De Vries et al., 2006). Fungal

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hyphae also entangle soil particles and produce organic binding agents, thereby promoting soil aggregation and providing physical protection of SOM (Tisdall et al., 1997). Moreover, fungal residues have been found to degrade slower than bacterial residues (Martin and Haider, 1979). These findings prompted the use of the fungal/bacterial (F/B) biomass ratio as an indicator of sustainable agro-ecosystems with potential for soil C storage (Bardgett and McAlister, 1999; Bailey et al., 2002).

Several studies found that reduced tillage (RT) practices increase F/B ratios, presumably because they decrease the disruption of hyphal networks, increase soil moisture contents and alter the distribution of crop residues (Frey et al., 1999; Hendrix et al., 1986). These results lead to the hypothesis that RT practices may promote C storage by inducing a functionally dominant role for fungi in SOM dynamics (Six et al., 2006). However, total fungal and bacterial biomasses alone do not fully capture microbial community characteristics relevant to SOM dynamics for several reasons.

Firstly, total fungal biomass comprises the two functional subgroups of free-living saprotrophic fungi (SF) and arbuscular mycorrhizal fungi (AMF). The AMF form symbiotic relationships with plant roots, in which plants supply fungi with carbohydrates, and fungi supply plants with nutrients and water. The SF on the other hand are not directly involved in plant nutrition. Soil microarthropods may feed preferentially on SF hyphae rather than on AMF (Klironomos and Kendrick, 1996), suggesting AMF have a relatively slow turnover time. AMF are also thought to interact with beneficial rhizosphere microorganisms including free-living N fixing bacteria and general plant growth promoting rhizobacteria (Gosling et al., 2006). For these reasons, SF and AMF play different roles in SOM dynamics. However, few studies have examined management effects on the composition of the fungal community (e.g. Bradley et al., 2006).

Secondly, the use of F/B biomass ratios implies that the role of fungi and bacteria in SOM dynamics is mostly determined by their abundance. But it is their growth rate and biomass production, rather than the biomass of different microbes *per se*, that controls their input into the energy channels of the soil food web (Bailey et al., 2002; Rousk and Bååth, 2007a). Yet to our knowledge, no studies have made a distinction between tillage effects on fungal and bacterial growth.

Finally, recent evidence suggests that microbial residues are stabilized in the soil (Glaser et al., 2004). Not only do these residues represent a SOM pool larger than living microbial biomass (Guggenberger et al., 1999); as microbial residues have a slower turnover time than microbial biomass, they could potentially serve as a time-integrated biomarker for microbial composition (Glaser et al., 2004). To approach a more complete understanding of how soil tillage affects SOM dynamics, we therefore need to consider its effect on the concentration, production *and* stabilization of microbially derived organic matter.

Fungal and bacterial contributions to the soil microbial biomass can be determined by a range of methods (Joergensen and Wichern, 2008). Phospholipid (PLFA) and neutral (NLFA) fatty acids, which are main components of the cell membrane and storage lipids (mainly in eukaryotic organisms), respectively, are often analyzed to characterize soil microbial communities. The PLFA 18:2ω6,9 has been used as a biomarker for SF and ectomycorrhizal fungi, whereas NLFA 16:1ω5 indicates storage compounds of AMF (Olsson et al., 1997). Fungal and bacterial biomass can also be estimated through microscopic assessment of hyphal lengths and bacterial cell volumes (Bölter et al., 2006). However, AMF and SF cannot easily be distinguished by microscopy (Sylvia, 1992). Ergosterol, the predominant sterol in fungal cell wall membranes, is also often used as a fungal biomarker. As ergosterol is not present in AMF, it indicates the presence of SF and ectomycorrhizae only.

Bacterial growth rates in soil have been estimated by measuring the incorporation of ^{3}H-leucine into bacterial proteins (Bååth, 1994). More recently, Bååth (2001) introduced the acetate-in-ergosterol incorporation technique to determine fungal growth rates as well. Finally, microbial residues can be estimated by quantifying the soil amino sugars glucosamine, galactosamine and muramic acid (Amelung, 2001). As these sugars occur in different ratios in fungal and bacterial cell walls, their relative abundance can be used to distinguish between fungal and bacterially derived residues (Joergensen and Wichern, 2008; Van Groenigen et al., 2007).

Here we aim to determine the impact of RT practices on the soil microbial community in a winter wheat field by simultaneously measuring biomass, growth rates and residues of both fungi and bacteria. By comparing biomass analyses that targeted SF and/or AMF, we could distinguish between responses of these two fungal subgroups. We also measured aggregate size distribution to obtain an index of soil stability. We hypothesized that RT increases the biomass and growth rate of fungi relative to bacteria, and that treatment effects on fungi and bacteria are reflected in the concentration of their respective residues.

2. Materials and methods

2.1. Site description

In the autumn of 2000, sixteen 27 × 30 m plots were established in a winter wheat (*Triticum aestivum* L.) field at Teagasc Crops Research Centre near Carlow, Ireland. Prior to the start of the field experiment, the site had been under conventionally tilled cropland for 8 years. Before that, it had been under permanent pasture for 10 years. The soil at this site is a haplic luvisol with a sandy loam texture (72% sand, 23% silt, 5% clay) and a pH of 6. Mean annual precipitation and temperature are 824 mm and 9.4 °C. Fertilizer N was supplied in the form of calcium ammonium nitrate at the rate of 200 kg N ha^{-1} y^{-1}, divided over three splits during the growing season. Further details of the experimental site can be found in Fortune et al. (2005).

Half of the sixteen plots were conventionally tilled (CT). The CT plots were ploughed to a depth of 20–25 cm, usually in late September. Ploughing was followed by secondary cultivation using a rotary power harrow (Lely Roterra), working to a depth of approximately 10 cm, immediately prior to sowing. The other half of the plots was subjected to an RT treatment, consisting of shallow non-inversion tillage using a single pass of a tined stubble cultivator (Horsch Terrano FX) at a depth of 7–10 cm, carried out in August soon after harvesting the previous wheat crop. Following stubble cultivation weeds and volunteer cereals were allowed to regrow for three to six weeks on the RT plots before application of the herbicide Arelon. Available data suggest that in the long term, the active ingredient of Arelon (Isoproturon) has no differential effects on the growth or biomass of fungi and bacteria (Nowak et al., 2006; Widenfalk et al., 2008). All plots were sown with a cultivator type drill with disc coulters (Vaderstad Rapide) on the same date in the first half of October. The actual date of secondary cultivation on the CT plots and sowing on all plots depended on soil moisture. After harvest, straw (crop residue) was chopped and incorporated in half of the CT and RT plots, by the ploughing and stubble cultivation operations, while it was baled and removed from the other half. This study focuses on the plots with straw incorporation.

2.2. Sample collection and processing

In May 2008, four soil cores (diameter 5.6 cm) were collected per plot at two depths (0–5 cm and 5–20 cm). In July 2008, two additional cores were taken per plot to determine soil bulk density.