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Bacterial community response to tillage and nutrient additions in a long-term wheat cropping experiment

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ABSTRACT

The effects of agricultural management practices on bacterial community structure and function are not well defined, yet are of concern for long-term soil resilience. In this study, soil microbial biomass, bacterial community structure (determined by TRFLP), and function, (determined by enzyme assays and Biolog assays), and soil physicochemical properties were investigated in a wheat cropping system subjected to long-term tillage (20 years) and short-term nutrient addition treatments. Samples were taken over a full cropping cycle. Tillage, nutrient addition, and time all significantly affected bacterial community structure (rDNA and rRNA), which showed considerable shifts across the sampling period. Microbial biomass and Biolog profiles changed significantly with time, but were not affected by treatment. With regard to specific enzyme assays, there were significant main effects of treatment and time on glucosidase, phosphatase and phenol oxidase enzyme activity, while for cellobioside and peroxidase, treatment and time had significant interactive effects. For the hydrolases significant effects were observed between nutrient treatments, while for the oxidases they were observed between tillage treatments. Overall, however, we found little evidence of major detrimental long-term effects of tillage on the soil bacterial communities or their important functions in the dryland wheat system studied. The bacterial communities showed both long and short-term trajectories that could be disentangled with appropriate experimental design. Concerns over significant long-term detrimental impacts of tillage on the soil bacterial communities appear unfounded, at least under systems similar to those studied here.

1. Introduction

Increasing global demand for food is leading to the intensification of agricultural practices and increasing demands on natural soil resources. As the primary organisms responsible for biogeochemical cycling, soil microbial communities catalyse a range of processes, such as nutrient cycling, which are important to the productivity and sustainability of soil ecosystems. While the direct relationships between microbial community structure and function are often difficult to elucidate, it is both the structure and function of soil communities that determine their response to perturbation and, therefore, the sustainability of changing land-use practices.

Intensification of land-use, and increasing recognition of the need to improve sustainability in agricultural practices, has led to the widespread adoption of minimal impact processes, including no-till (NT) cropping systems. NT practices have been adopted in the belief that they improve soil quality and crop yield potential. Specifically, NT has been shown to increase microbial biomass (MB) (Helgason et al., 2010), improve soil carbon (Lal et al., 2003), increase mineralizable N (Spargo et al., 2011), increase soil moisture (Ma et al., 2008), increase enzyme activities (Alvear et al., 2005) and reduce greenhouse gas emissions (Six et al., 2004). In contrast, other studies have shown that NT effects on moisture (Kanwar, 1989), microbial activity and biomass carbon (Calderon et al., 2001), mineral N (Gomez-Rey et al., 2012) and green house gas emissions (Bayer et al., 2012) are minimal or short-lived. The time over which assessments of NT efficacy are conducted are also of importance, with Calderon et al. (2001), for example, finding short-lived changes and Six et al. (2004) showing that changes are not evident in the short-term, but become evident after many years in some systems. Further, the efficacy of NT systems to sequester soil carbon is also the subject of considerable debate. It has been shown that early studies demonstrating sequestration did so because of methodological flaws, and carbon was not sequestered, but merely redistributed throughout the soil profile (Baker et al., 2007). It has also been shown that soil C input differences are responsible for

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differences sometimes seen in C sequestration between conventional tillage (CT) and NT soils (Govaerts et al., 2009; Virto et al., 2012), rather than tillage technique. It is clear, then, that there is a wide literature on the effects of NT (of which several examples have been cited above) and that while NT may be efficacious in reducing erosion, it does not necessarily result in improved soil quality, yields or sustainability (kirkegaard, 1995). Further, the conversion of native ecosystems to agriculture is a major disturbance and it has been suggested that it is this process, rather than specific tillage practices which are relatively minor in disturbance, that is the primary cause of soil degradation (DuPont et al., 2010).

Nutrient addition has also been shown to affect soil microbial community structure and function both positively and negatively, including effects on C and N-cycling (Khan et al., 2007) and enzyme activities (Saiya-Cork et al., 2002; Sinsabaugh, 2010; Sinsabaugh et al., 2004). The fact that studies have shown both detrimental and enhancing effects of nutrient amendment illustrates the complexity of relationships between structure and function in continually changing environments such as agricultural soils.

Although it is generally agreed that microbial diversity in soils is crucial to soil function, few studies have directly linked diversity and function, thus we currently have little ability to predict the outcome of specific management interventions. An understanding of how microbial communities respond to different agricultural practices and perturbations is, therefore, important in seeking to maximise the health and sustainability of soil resources. Advances in DNA-based technologies allow microbial community structure and shifts to be inferred from a variety of methods, of which community fingerprinting allows appropriate ecological replication. Fingerprints may also be derived from what can be considered the total community, via DNA, or from the active community, via RNA (Mengoni et al., 2005). In some sense it could be argued that the former represents the larger "standing community" and its capacity to deal with change (i.e., longer-term resilience), while the latter reflects current conditions and the community's responses to short-term environmental change (e.g., resistance to anthropogenic disturbance).

While the direct relationship between community structure and function is unclear, it is certain that functional diversity is important in maintaining the sustainability of soil resources. Soil function may be assessed by monitoring specific biogeochemical transformations (e.g., N cycle) or by monitoring, more generally, substrate use profiles and enzyme activities. The latter offers the ability to monitor general soil functional changes, but not the ability to comment on specific processes of interest and rate changes. Previous work has shown that substrate utilization patterns may change with different tillage regimes (Lupwayi et al., 1998) or that they may not (Bissett et al., 2011).

Soils are very heterogeneous, both spatially and temporally (DeAngelis et al., 2011; Grayston et al., 2001; Schmidt et al., 2007), and this variation must be factored into experimental designs addressing specific questions regarding treatment effects. Biogeochemistry and community structure of soils and sediments may change predictably on both long (seasonal) (Caporaso et al., 2012; Cruz-Martinez et al., 2009; Fuhrman et al., 2006) and short (hours) (DeAngelis et al., 2011) timescales. In order to understand long term trajectories it is, therefore, necessary to have an understanding of any short term fluctuations that may affect perceptions of the system's current state in relation to the longer term changes being assessed. This is especially true in systems that exhibit any degree of hysteresis (Schoon et al., 2010).

To study the effects of management practices on soil bacterial community structure and function we sampled soils at multiple time points from a long-term agricultural field trial primarily investigating tillage effects within a grain cropping environment (Kirkegaard et al., 1994). We investigated NT and CT treatments in place for over 20 years as examples of least and most soil disturbance, as well as the shorter-term effects of nutrient enrichment with crop residue incorporation, by assessing soil physicochemistry, bacterial community structure as determined by both 16S rRNA gene and 16S rRNA TRFLP fingerprints, microbial biomass C and N and bacterial community function as assessed by community level physiological profiles and specific extra-cellular enzyme assays. This polyphasic approach was employed to investigate changes in both active and standing bacterial community structure and function in response to both time of sampling during an annual cropping cycle and agricultural land-use treatment. Our aim was to determine if these management practices changed soil bacterial communities or function in ways likely to threaten the long-term productivity of the soil.

2. Materials and methods

2.1. Experimental design

A factoral design, comprising 3 factors (treatment, time, plant presence), was used to investigate the effects of management practices on soil bacterial properties. Three treatments were arranged in a randomised block design with four blocks of individual plots measuring 30 m × 6 m. This study utilised two of the original treatments imposed in 1990 and one treatment imposed in 2007. The two oldest treatments comprised the original Stubble Incorporation (Incorp) treatment, in which an offset disk harrow was used to incorporate stubble to a depth of 15 cm after crop harvest (February/March) each year, and the Stand Stubble Direct Drill (DD) treatment, in which stubble was allowed to remain standing without disturbance. The new treatment (Incorp + N) comprised the Incorp treatment plus the addition of extra nutrients at the time of stubble incorporation (March, nutrients: residue 10 t/ha, 25.6 kg/ha N, 21.5 kg/ha P, 18.8 kg/ha S) to achieve a C:N:P:S ratio thought to be required for maximum soil C sequestration (Himes, 1998) and the conversion of sequestered C into humus (Kirkby et al., 2011). Time comprised 5 levels, chosen to approximate seasons, with a more intense sampling at the time of nutrient and residue incorporation (February–March) (Fig. 1). The final factor, plant presence, comprised two levels (on-plant (row) and off-plant (gap)), to test for the effect of growing wheat plants.

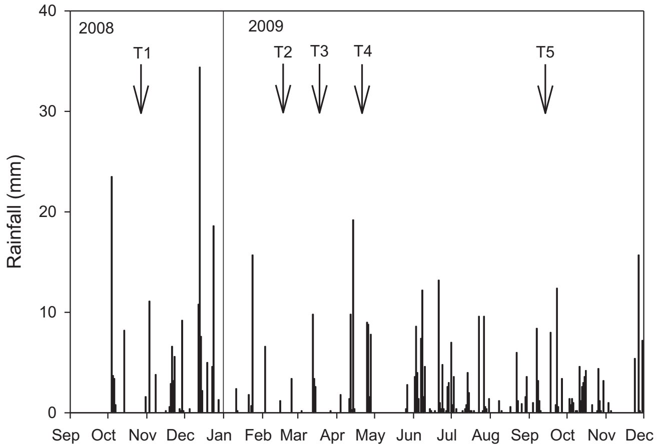

Fig. 1. Daily rainfall at experimental site over the study period. Average rainfall for the Harden area is approx. 600 mm, falling equally throughout the year (50 mm/month). Total rainfall for the 2009 was 340 mm. Sampling dates are indicated by arrows. Agricultural management at each sampling point was as follows: T1 = mature crop, T2 = crop residue incorporated and nutrients for Incorp + N treatment added, T3 = Pre-crop, T4 = early crop, T5 = grain filling stage.