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Temporal and seasonal change in microbial community structure of an undisturbed, disturbed, and carbon-amended pasture soil

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ABSTRACT

Disturbance and change to C inputs can alter microbial community structure and impact ecosystem function. Particularly in temperate regions, seasonal change also has an effect on microbial communities both directly through climate and indirectly through plant function. The temporal change in microbial communities of an undisturbed pasture, disturbed pasture (similar to a single tillage event) and pasture soil amended with two forms of particulate carbon were monitored over eight consecutive seasons after grass was reestablished. The soil microbial community was assessed by a DNA fingerprinting technique (terminal restriction fragment length polymorphism, TRFLP) of bacterial, fungal and archaeal communities, and also from phospholipid fatty acid (PLFA) analysis. The single disturbance had a significant effect on fungal microbial community structure (by TRFLP) and significantly decreased the fungal:bacteria ratio. Though the change was relatively small it persisted throughout the sampling period. Nitrate was also higher on the disturbed treatment providing evidence for the theory that changes to fungal:bacterial ratios can alter nutrient cycling and retention. Fungal communities were the most altered by the C amendments. While bacteria were also affected by the C amendments, seasonal change was a greater cause of variation in this community. Correlation to soil and climatic variables explained more of the total variability for PLFA (78% for all treatments) than bacterial (50%), fungal (35%) and archaeal (14%) restriction fragments. Most climate and soil variables explained significant variation for seasonal patterns in the multivariate community structures but measurements of soil moisture were important for all communities while pH was relatively more important for bacteria, temperature for fungi, and soil C:N ratio for archaea. Autumn was particularly distinct from other seasons for bacteria (less so for the fungal community) and although there was seasonal change in pH suggesting pasture management was a factor, the significant correlation of other soil characteristics suggests that plant physiological changes (most probably root exudates) also played a significant role. The large change in the saprotrophic fungal community due to the particulate C addition but minor seasonal change would tend to suggest that the fungal community may be more responsive to changes in litter inputs rather than root exudates while the reverse is true for bacteria.

1. Introduction

The composition of the microbial community can have functional significance on ecosystem processes (Strickland et al., 2009). Some studies have shown that changes in land use result in changes to microbial community structure (e.g. Bossio et al., 1998; Macdonald et al., 2009; Drenovsky et al., 2010), but other studies (Lauber et al., 2008), have suggested that edaphic characteristics are a more important factor. Among the impacts of changing land use on soils are disturbance such as tillage, which modifies microbial habitat at the aggregate scale, and alterations in the quality and quantity of C inputs through addition of nutrients and changes to composition and production of vegetation.

There is a body of literature on effects of tillage and balance between fungal and bacterial ratio in tillage system (e.g. Frey et al., 1999; Feng et al., 2003; Helgason et al., 2010a). Strickland and Rousk (2010), however, suggest that changes in fungal communities and especially fungal to bacterial ratio may not be as clear cut as has been suggested. Both the method of determining changes to

Abbreviations: MBC, microbial biomass carbon; PLFA, phospholipid fatty acid analysis; TRFLP, terminal restriction fragment length polymorphism.

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fungal and bacterial communities, and the covariance of factors such as moisture (Frey et al., 1999) may play a role in determining change in microbial community structure.

Altering carbon (C) inputs into soil has been shown to directly alter microbial community structure (Nemergut et al., 2010; Prevost-Boure et al., 2011). The indirect effects of seasonal change on plant growth, however, can also affect the C inputs into the soil through litter/root decomposition and root exudates. While temporal and seasonal changes in microbial community structure (particularly for the bacterial community) have been noted (Schutter et al., 2001; Griffiths et al., 2003a,b; Kennedy et al., 2005), they have arguably been less studied than spatial and treatment effects.

We utilized dairy pasture plots that had been disturbed (similar to a single tillage event) and amended with two forms different forms of particulate C (sawdust or coarse woody mulch) to quantify and contrast these effects with temporal (and seasonal) changes in different aspects of the microbial community structure. Treated plots were measured over eight consecutive quarterly measurements after the disturbed and C amended plots had been returned to full grass cover.

Both a DNA fingerprinting method (terminal restriction fragment length polymorphism (TRFLP) of the bacterial, fungal and archaeal communities) and phospholipid fatty acid (PLFA) analysis were used to characterize microbial community structure. Climate (temperature, precipitation and calculated soil water storage) and soil (total C and nitrogen (N), laboratory net N mineralization, pH and basal respiration) data were used to correlate climatic and edaphic variables with changes in microbial community structure over time.

We predicted that the disturbance would decrease the fungal community (and the fungal to bacterial ratio) and that incorporation of the carbon substrates would have measurable effects on microbial community structure. More specifically, we predicted that the response of the different components of the microbial system would vary in magnitude to the treatment effects, and that temporal and seasonal patterns of the different components of the microbial community structure would differ.

2. Material and methods

2.1. Site and experimental design

The study site was located on a dairy farm in the central Waikato of the North Island of New Zealand (37° 47′S, 175° 19′E). The region has a temperate climate with mean annual precipitation of 1250 mm y^{-1}, mean summer temperature of 23.8 °C and mean winter temperature of 13.6 °C. Summer (January–March) is generally the driest part of the year and droughty conditions often occur, but the maritime influence on climate can result in significant precipitation throughout the year. Although frosts can occur in winter, the soil seldom freezes and grass growth can occur through winter, though at a much slower rate than spring/summer when soil temperatures are warmer.

The soil was Horotiu sandy loam (Typic Orthic Allophanic Soil, New Zealand classification; Vitric Hapludand, US Soil Taxonomy). The Horotiu soil is derived from volcanic ash, and is typically high in carbon content and well drained. Pastures consisted of a ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) mix. Stocking rate was 3.0 cows ha^{-1} and the plots received 220 kg N ha^{-1} y^{-1} in the form of urea during the study applied in split applications of approximately 36.8 kg N ha^{-1}.

Full details of the study site and layout can be found in Stevenson et al. (2011). In brief, the study was set out in a randomized complete block design with five blocks and four treatment plots (5 × 5 m) in each block. Treatments were initiated in September of 2007 and consisted of: (1) control pasture; (2) a disturbed treatment (to control for the effects of soil disturbance associated with amended treatments); (3) a sawdust-amended treatment; and (4) a coarse woody mulch-amended treatment. The two organic amendments were picked largely to give slightly different nutrient content and size class. The sawdust was obtained from a *Pinus radiata* mill and particle size was generally less than 1 mm diameter. The coarse woody mulch was obtained from a local tree-cutting service where woody material (bark, limbs, and trunks) had been put through a shredder.

In the C-amended treatments (referred to as amended treatments) approximately 55 Mg ha^{-1} (dry wt.) of the amendment was rototilled into the A horizon of the soil to a depth of approximately 15 cm. For the disturbed control, the soil was rototilled to a similar depth but no amendment was added. The amended and disturbed plots were reseeded with a ryegrass/clover mix that matched surrounding pasture and fenced off to restrict grazing until grass cover had reached 100%. Fencing was removed and grazing was allowed on all plots for 2 months before the initial sampling. The elapsed time from disturbance to the start of monitoring was approximately 6 months.

Temperature, precipitation and evapotranspiration data were used from the nearby NIWA Ruakura weather station (approximately 6 km from the site). The evapotranspiration data allowed the calculation of soil moisture storage. A value of 150 mm is a typical saturation level for this soil type. Moisture stress was defined as soil moisture storage <90 mm. Although it is possible that C additions may have altered saturation and moisture stress values, there were insufficient data to set separate levels for each treatment, so the values were kept constant between treatments.

2.2. Sampling and soil analytical procedures

Beginning in late February 2008, approximately ten 2 cm × 10 cm soil cores were aseptically collected every 3 months from each plot and bulked to analyze for soil chemical and biological measurements. Total C and N were analyzed by dry combustion on air-dried, sieved (<2 mm) and finely ground soils using a Leco 2000 CNS analyzer (St Joseph, MI, USA). Soil pH, soil nitrate-N and basal respiration followed the procedure outlined in Blakemore et al. (1987). In brief, soil pH was measured using a combination electrode from a 1:2.5 soil to water extract. Soil nitrate was measured from a 2 M KCl extract. Basal respiration rate was measured as the increase in CO_2 content during 7 days incubation at 25 °C and field moisture content.

Microbial C was measured on field-moist samples using the fumigation-extraction procedure (Wu et al., 1990) with soil adjusted to 60% water-holding capacity. The k-factor used for converting extractable C flush to microbial C was 0.41 (Sparling and Zhu, 1993). Net N mineralization was measured on freshly collected soils by subtracting final from initial 2 M KCl extractable ammonium and nitrate from soil incubated aerobically at −5 kPa moisture content for 56 days at 25 °C. Due to a sampling mishap, samples from the second winter sampling (August, 2009) were not analyzed for microbial biomass C and N mineralization, and are recorded as missing data.

Bulk density was calculated from five 1.5 cm diameter cores taken from each plot during each sampling. Previous work on these plots had indicated that bulk density did differ for the amended treatments (see Stevenson et al., 2011), but preliminary analysis indicated little difference in results whether soil characteristics were analyzed on a gravimetric or a volumetric basis. Gravimetrically expressed data were used as they generally had less variation over time.

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