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Microbial community structure and function respond more strongly to temporal progression than to the application of slurry in an Irish grassland

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ABSTRACT

The application of slurry to grassland for fertilization purposes is common practice, but its effect on the soil microbiota is mostly overlooked. This study investigated the short term response of the functionality and composition of the soil microbiome to slurry application. A 180 m^2 field was divided into 36 plots. Slurry was splash-plate applied at a rate of 30 t ha^{-1}. Sampling was conducted 5, 30 and 65 days after application. The functionality of the soil microbial community was examined using assays on 8 carbon cycling enzymes as well as basal respiration analysis. Microbial community structure was analysed via bacterial 16S rRNA gene and fungal internal transcribed spacer region terminal restriction fragment length polymorphism. Bacterial and fungal abundance was determined via quantitative PCR aiming at the same genetic targets. Furthermore, microbial biomass carbon and nitrogen were quantified. A significant increase in enzymatic activity with slurry treatment was reported on days 5 and 65, indicating a sequential response of the microbiota to slurry-derived carbon with the utilization of labile carbon on day 5 and the more stable carbon on day 65. This activity seemingly resulted from the microbial demand for N. In contrast, T-RFLP revealed that only bacterial community structures on day 5 were significantly affected by slurry application, all other bacterial and all fungal communities were not significantly altered by slurry. However, bacterial and fungal community structures, microbial biomass carbon and basal respiration significantly responded to temporal progression (day 5, 30 and 65). These findings suggest that soil microbial communities are responding to slurry applications via enhanced microbial activity but their structure remains largely unchanged with temporal progression having a greater impact.

1. Introduction

Pasture-based livestock production is the predominant agricultural activity in Ireland and is common in temperate climates worldwide. Animals are typically kept out on pasture between March and November and are then over-wintered indoors. This is usually to protect the soil from trampling and compaction during the wetter winter months. This over-wintering produces a large quantity of slurry and its application back onto the land is an imperative practice for the recycling of nutrients in Irish agricultural systems (Holden et al., 2004).

Despite the critical role the soil microbiome plays in the cycling of slurry derived nutrients, in particular carbon (C), the soil-slurry-microbiota interactions is largely unknown (Harris et al., 2011). Slurry derived C is typically classified into labile and recalcitrant fractions. These fractions are differentially incorporated into the soil matrix (Rochette et al., 2000). The labile C fraction (glucose, xylose etc) is incorporated within a few days after slurry application while the more recalcitrant C fraction (cellulose, lignin) is incorporated over a period of weeks (Dungait et al., 2009; Rochette et al., 2000). This dynamic will correspondingly affect the soil microbial community. It is assumed that initially there will be a large increase in microbial (particularly bacterial) biomass as the labile C fraction is utilized as an easily metabolizable energy source (Fontaine et al., 2003). As this energy source becomes exhausted, the microbial community will subsequently use the recalcitrant C component of the slurry and potentially the soil organic carbon (SOC) at increased rates (Bol et al., 2003b; Bourdin et al., 2010; Kuzyakov and Bol, 2006).

The effect of slurry application on bacterial and fungal community structure is little understood and little reported in the literature and this is especially the case for field applications (Harris et al., 2011). The effect of cattle slurry on bacterial community structures in soils under laboratory conditions has been recently reported, where DNA-

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fingerprint shifts were seen (Abubaker et al., 2013). However, these effects were at application rates (66 g kg^{-1}) which exceeded agricultural practices. Consequently, the effect of in-field slurry addition at conventional application rates for a temperate grassland (30 t ha^{-1}; Bourdin et al., 2014) on the soil bacteria and fungi is still largely elusive. Harris et al. (2011) synergised the varying interconnected aspects of the soil-slurry interaction and the consequences for biogeochemical cycling. They hypothesized that the site input history (i.e. N, P and K fertilizer regime, previous slurry application) will influence the composition of the soil microbial community as well as its functional capacity. Long-term inputs into a site will alter the configuration of the microbial community, which will be an important factor in determining the immediate microbial response to slurry input. Indeed, it has been reported that in intensively managed Irish grasslands soil biological communities are largely insensitive to inorganic fertilizer application and more sensitive to temporal effects e.g. seasons (Massey et al., 2016). Determining the magnitude of disturbance on the soil microbial diversity and function resulting from slurry application at agricultural practice is an important factor to evaluate the effect of slurry to soil health.

The experimental aim of this study was to determine the short term response of the composition and functionality of the soil microbial community to the addition of slurry on a field scale using a conventional application rate. Additionally, it aimed to ascertain whether slurry application or temporal progression had the more pronounced effect on the dynamics of the soil microbial community.

2. Materials and methods

2.1. Field site set-up

The experimental field site was located at the experimental research station at Teagasc Johnstown Castle, Wexford, Ireland (52°18′N; 6°30′W). The site is a well-drained coarse loam over fine loam, classified as Haplic Cambisol (WRB, 2006), with an established (> 10 yr) and uniform ryegrass (*Lolium perenne*) sward. The average rainfall and temperature at this site (averaged over 25 years 1978–2003) were 1044 mm and 10 °C (Bourdin et al., 2014). In 2009, the site was used in experimental field trials regarding slurry application (Bourdin et al., 2014). From 2010–2013, the site was used for the light grazing of livestock. In 2010, there were two applications of N (in the form of calcium ammonium nitrate, CAN) at 27 kg h^{-1} (May) and 20 kg h^{-1} (July). In September 2012, the site received 14.18 kg h^{-1} of N (CAN). In April 2013, 30.3 kg h^{-1} of urea was applied to the site, while the following September it received a further 27.63 kg h^{-1} of N (CAN).

In May 2014, grass was cut to 5 cm height on a 180 m^2 area which was divided into 36 plots (2 m x 1.5 m with a 0.5 m gap between each; Bourdin et al., 2014). The site was left for 1 week to allow time for the soil microbial community to stabilize after grass cutting. Plots were set out in a randomized block design (supplementary Fig. S1). On the 9th of May 2014, slurry was spread simulating the splash-plate method at a rate of 30 t ha^{-1} on 18 of these plots (Bourdin et al., 2014). The applied slurry (8.18% dry matter; 7.61 pH; 41.80% C; 2.50% N; 16.72C/N; 49.5 ppm PO_4^{3-} and 11.1 ppm SO_4^{2-}) was collected from beef cattle fed by a ryegrass silage. The remaining plots received no slurry and acted as controls. The variable in this experiment was the time after slurry addition that the plots were sampled. There were three sampling times: 5, 30 and 65 days after slurry application. Sampling times were selected on the basis of the findings of a slurry experiment conducted by Bourdin et al. (2010). At each sampling event, six of the slurry-amended plots were sampled in conjunction with six control plots.

2.2. Sampling strategy

Each plot was sampled in five separate points in a 'W' pattern using a 7 cm Dutch auger to a depth of 10 cm. Approximately 600 g of soil was collected from each plot and placed in sterile plastic bags. The auger was disinfected after being washed thoroughly with 70% (v/v) ethanol and sterile water between samplings. These were stored on ice until transported back to the lab (max. 2 h) where samples were stored at 4 °C. Fresh soil samples were stored for no more than 1 week before various analysis were undertaken. In the lab, samples were mixed and sieved (2 mm) and a sub-sample was frozen at −80 °C for subsequent molecular work. All analyses requiring fresh soil were done within 10 days of collection.

Table 1
Averages (Avg.) and standard deviations (±) of particle size distribution, (% of course and fine sand, silt and clay), pH, C and N content from the first 10 cm of experimental site (P1-P10; n = 10). Data collected in April 2014 prior to the establishment of experiment.

Environmental variable	Avg.	±
% Course sand (2–0.2 mm)	32.1	2.64
% Fine sand (0.2–0.05 mm)	26.1	3.28
% Silt (0.05–0.002 mm)	27.2	2.66
% Clay (< 0.002 mm)	14.6	1.84
pH	5.27	0.12
% C	3.46	0.38
% N	0.33	0.03
C/N	10.39	0.21
ppm PO_4^{3-}	8.69	4.07
ppm SO_4^{2-}	10.96	2.69

2.3. Edaphic and environmental properties

Prior to the start of the experiment (April 2014), soil samples (10 cm depth) were taken from around the perimeter of the designated experimental area to determine particle size distribution (Supplementary information SM1), pH, total % C and % N content as well as levels of water extractable phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) (Table 1). Variables were measured as per the procedures developed by the Irish SIS laboratory protocols (Massey et al., 2014). Briefly, gravimetric soil water content was determined on 5 g of sieved (2 mm) soil which had been dried in a fan-oven at 105 °C for 24 h. Soil pH was determined using a glass electrode in a 1:2.5 soil to dH_2O solution. The total % C and % N was determined using a LECO Truspec CN analyser (LECO Corporation, Saint Joseph, MI). Phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) was determined via ion chromatography using a Dionex ICS1100 with an AS23 column and a carbonate mobile phase as recommended by the manufacturer (Sunnyvale, CA)(Fox et al., 2016). All measured parameters (with the exception of particle size) were also conducted on each experimental plot.

2.4. Enzymatic assays of C-cycling enzymes

Extra-cellular enzymatic activity was determined on 8 different fluorogenic model substrates; 4-Methylumbelliferyl (4-MUF)- α-D-mannopyranoside, 4-MUF-α-L-arabinopyranoside, 4-MUF- β-D-Cellobioside, 4-MUF- β-D-xylopyranoside, 4-MUF-N-acetyl- β-D-glucosaminide, 4-MUF- β-D-galactopyranoside, 4-MUF- β-D-glucopyranoside and 4-MUF- α-D-glucopyranoside (all Sigma Aldrich, St. Louis, MO). These were used to measure the enzymes β-glucosidase, β-N-acetyl-glucosaminidase, β-xylosidase, cellobiosidase, α-glucosidase, β-galactosidase, α-arabinosidase, and α-mannosidase. 5 g of fresh soil was placed into 50 ml of sterile dH_2O and enzymes were extracted by shaking on a Gyratory shaker (New Brunswick Scientific) at 150 rpm for 10 min, followed by centrifugation at 750 rpm for 10 min at 4 °C. 200 µl of this supernatant was pipetted into individual wells of black microtiter plates (VWR, Radnor, PA). The plates had initially received 10 µl of MOPS (3-(N-morpholino) propanesulfonic acid sodium salt) buffer (pH 7.4) as well as 40 µl of the analogous MUF-substrate (at a final concentration of 50 µM). For standard curve calculations, MUF-salt (4-methylumbelliferone) in four concentrations was added (Hendriksen