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Short communication

Impacts of surface-applied residues on N-cycling soil microbial communities in miscanthus and switchgrass cropping systems

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ABSTRACT

The production of biomass crops such as the perennial grasses (PGs) miscanthus (*Miscanthus* spp.) and switchgrass (*Panicum virgatum* L.) has increased considerably. The repeated annual harvest of aboveground PG biomass removes organic inputs from the soil and may influence soil health and soil microbial communities, which drive terrestrial nitrogen (N) cycling, influencing the ecosystem services provided by these feedstock systems. Our objective was to assess soil bacterial N-cycling communities as influenced by the return or removal of aboveground plant biomass (residues) to soils in PG biomass feedstock systems at different N fertilization (0 or 160 kg N ha^{-1}) rates. Soil was collected from a field trial and quantitative PCR was used to enumerate bacterial 16S rRNA and denitrifying (*nirS* and *nosZ*) genes and transcripts. Denitrifier gene expression (*nirS* and *nosZ*) was significantly higher in N-fertilized compared to unfertilized plots, indicating that applying fertilizer in these systems may shift the activity of the denitrifying populations and possibly lead to associated N losses with no return in yield. Returning biomass residues resulted in significantly higher *nosZ* transcripts than in soils with residues removed but did not influence *nirS* gene expression. The removal of plant residues in these systems may influence the activity of the nitrous-oxide reducing microbial community, resulting in potential changes in the ecosystem services moderated by soil microbial communities, which may need to be incorporated into future soil health assessments of bioenergy feedstock systems.

1. Introduction

Plant residues provide surface cover and alter soil microhabitats, moderating soil temperature, moisture and gas diffusivity (Blanco-Canqui and Lal, 2009), which may shift microbial metabolic niche diversity and ecosystem functioning (Orr et al., 2015). Reducing greenhouse gases (GHGs), such as N_2O is a core goal of bioenergy production (Thomas et al., 2013). Studies have shown that amending soil with plant residues with high C:N ratios, such as PG biomass, can enhance N immobilization, reducing N loss and N_2O emissions (Huang, 2004; Miller et al., 2008; Muhammad et al., 2010). This indicates the repeated annual removal of PG biomass may be detrimental to soil health and environmental sustainability (Blanco-Canqui and Lal, 2009) due to the removal of organic inputs from the system.

Measuring N_2O directly within cropping systems associated with large biomass yields, such as biomass feedstocks, is difficult with chamber methods. Within field studies including multiple field treatments, micrometeorological measurements of N_2O flux are not possible. Alternatively, relative abundances of denitrification genes and transcripts can be measured to assess a soil's potential to produce or consume N_2O via denitrification. These targets may represent a proxy of relative N_2O emission potential (Butterbach-Bahl et al., 2013; Hallin et al., 2009; Morales et al., 2010; Petersen et al., 2012; Philippot et al., 2002), and previous studies have shown changes in denitrifier community sizes and gene expression to be correlated with both denitrification process rates (Hallin et al., 2009; Wu et al., 2012), and *in situ* N_2O fluxes (Németh et al., 2014; Thompson et al., 2016a).

Within PG feedstock production, management practices for increased yields and improved biomass qualities for various downstream uses have been investigated (Amougou et al., 2011; Sokhansanj et al., 2009); however, research on the effects of biomass removal and N fertilization on denitrifier communities in PG systems and their associated potential for N_2O emissions is scarce (Mao et al., 2013, 2011; Thompson et al., 2016b). Previously, conflicting results have been found in assessments of denitrifier communities under bioenergy crops. For instance, Mao et al. (2011) observed a gradual differentiation in N-cycling community structure between annual and perennial bioenergy crops, whereas further study by the same researchers indicated site-to-

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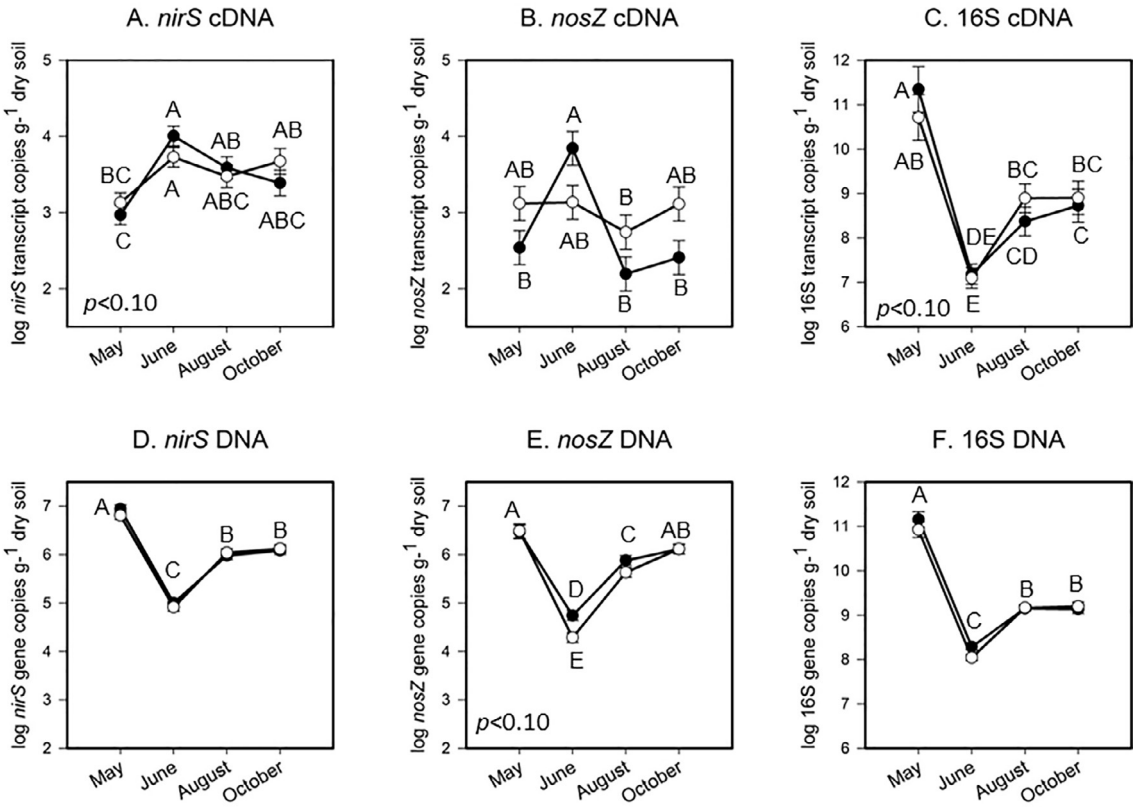

Fig. 1. Mean log gene copies and transcripts (*nirS*, *nosZ* and 16S rRNA) (g^{-1} dry soil ± SE) in PG systems at 0 and 160 kg N ha^{-1} (n = 36) averaged over residue management at the Elora Research Station over time. Filled symbols = MS, open symbols = SG. Different letters within panels indicate significant differences according to a post-hoc Tukey's test ($p < 0.05$).

site variation in N-cycling communities was larger than variation due to plant types (Mao et al., 2013). A parallel study (Thompson et al., 2016b) conducted at our study site in plots without residue manipulations, found that miscanthus produced significantly larger yields and supported larger N_2O-consuming (*nosZ*) communities than a traditional corn-soybean rotation, regardless of N fertilization rate. Presently, we chose to assess changes in both denitrifier community size and gene expression over a growing season under varied residue management in PG plots, to investigate whether residue management may impact short-term N cycling in these systems.

2. Methods

2.1. 1 Site description and soil analysis

A PG field trial was previously established at the University of Guelph Research Station in Elora, ON, Canada (43°38′46.73″ N and 80°24′6.66″ W) in 2008, as described in Thompson et al. (2016b). The trial was a split-split strip plot design with three replicates; the main plot (6.2 m × 26.0 m) factor was PG (miscanthus or switchgrass) and hand-broadcast urea fertilizer was applied in strips randomly within replicates in the spring of each year after trial establishment at0, 40, 80, and 160 kg N ha^{-1}. Three years after the main PG trial was established, residue subplots (1.25 m^2) were established in miscanthus ("MS") and switchgrass ("SG") plots in both 0 and 160 kg N ha^{-1} fertilizer strips. Residue subplots were assigned in a randomized complete block design within each PG crop × N rate plot; residue treatment subplots were either undisturbed (ca. 30% residue return via senescent leaf loss to soil, "U"), had residues removed from the soil surface (100% removal, "R−"), or had 100% of harvested mulched biomass (cut approx. 2 cm in length) returned to the soil surface ("R+") (Supp. Fig. 1).

Soil was sampled at 0–2.5 cm depth to examine the short-term effects of the return and removal of surface-applied residues on microbial communities at the soil surface over the growing season. Soil was sampled on May 9 (pre-N fertilization and pre-residue subplot establishment), June 27 (post-N and residue subplot establishment), August 16 and October 4, 2011. At each sampling time, 0.5 g of field-moist surface soil was collected along a transect (five subsamples of ca. 0.1 g each) from within each residue treatment subplot using aseptic scoopulas and immediately placed into sterile collection tubes containing 1 mL g^{-1} soil LifeGuard™ Soil Preservation Solution (MO BIO Laboratories, Inc.).

2.1.1. Quantification of denitrifying and total bacterial genes and transcripts

DNA and RNA were co-extracted according to the manufacturer's protocol using a RNA PowerSoil Total RNA Isolation Kit with a DNA Elution Accessory Kit (MO BIO Laboratories, Inc.). Reverse transcription of RNA to cDNA was conducted in triplicate using an Applied Biosystems® High-Capacity cDNA Reverse Transcription Kit (Life Technologies Corp.). Quantitative PCR (qPCR) assays were used to enumerate the total bacterial communities (16S rRNA), and communities of denitrifiers by targeting nitrite reductase (*nirS*) and nitrous oxide reductase (*nosZ*) gene and transcripts, using primer pairs and rationale for target choice as described in Thompson et al. (2016b). Duplicates of each target were run on an IQ5 thermocycler (Bio-Rad Laboratories, Hercules, CA, USA) with control plasmids and no-template controls which gave null or negligible values; further details of experimental design, methods, and statistical analyses are described in supplementary methods.

3. Results

There were no significant differences in soil moisture or soil NH_4-N