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 N₂O 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 N₂O 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 N₂O 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 N₂O 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 N₂O via denitrification. These targets may represent a proxy of relative N₂O 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 N₂O 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 N₂O 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-
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 N2O-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 at 0, 40, 80, and 160 kg N ha⁻¹. Three years after the main PG trial was established, residue subplots (1.25 m²) 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 scoops 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 NH₄-N
Tukey-ported a statistically significant soil-growth interaction (cDNA) of the total and denitrifying communities did not vary with N rate or PG crop species (Table 1); additionally, the potential difference in levels between any treatment factors, but both measures significantly increased from May to October sampling dates in SG soils (Supp. Table 1, Fig. 1); all other interactions were not significant (NS). Within target group columns, means followed by different bolded capital letters are significantly different between crop species (miscanthus “MS”, switchgrass “SG”); means followed by different capital letters are significantly different between residue treatments (R+ = residues returned, R− = residues removed, U = undisturbed), and means followed by different lower case letters are significantly different between N rates (0 and 160 kg N ha−1), according to a post-hoc Tukey’s mean comparison (p < 0.05). Table 1 Summary of ANOVA responses for mean gene and transcript copies (g−1 dry soil) at the Elora Research Station, as observed over the 2011 growing season under residue manipulation treatments, with corresponding means for significant direct effects (PG crop species, residue treatment and N rate).

<table>
<thead>
<tr>
<th>Direct Effects (Numerator df)</th>
<th>nosZ cDNA</th>
<th>nosZ DNA</th>
<th>nirS cDNA</th>
<th>nirS DNA</th>
<th>16S cDNA</th>
<th>16S DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue Treatment</td>
<td>F value†</td>
<td>p value</td>
<td>F value†</td>
<td>p value</td>
<td>F value†</td>
<td>p value</td>
</tr>
<tr>
<td>MS</td>
<td>4.12, ss</td>
<td>&lt; 0.05</td>
<td>1.04, ss</td>
<td>&lt; 0.05</td>
<td>1.64, ss</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SG</td>
<td>7.04, ss</td>
<td>&lt; 0.01</td>
<td>5.46, ss</td>
<td>&lt; 0.05</td>
<td>0.02, ss</td>
<td>NS</td>
</tr>
<tr>
<td>N rate</td>
<td>13.05, ss</td>
<td>&lt; 0.001</td>
<td>0.63</td>
<td>NS</td>
<td>7.91, ss</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Time</td>
<td>5.87, ss</td>
<td>&lt; 0.05</td>
<td>108.82, ss</td>
<td>&lt; 0.0001</td>
<td>14.7, ss</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time × PG crop</td>
<td>9.06, ss</td>
<td>&lt; 0.0001</td>
<td>2.65, ss</td>
<td>&lt; 0.10</td>
<td>2.15, ss</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td>All other interactions†</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

† F value subscript indicates the numerator and denominator degrees of freedom, respectively.
§ Interactions included all interactive effects for all model terms, including 2, 3 and 4-way interactions. The Time × PG crop interaction significantly impacted nosZ transcripts (cDNA) and genes (DNA, p < 0.10), nirS transcripts (cDNA, p < 0.10) and 16S rRNA transcripts (cDNA, p < 0.10) and genes (DNA) (refer to Fig. 1); all other interactions were not significant (NS). Within target group columns, means followed by different bolded capital letters are significantly different between crop species (miscanthus “MS”, switchgrass “SG”); means followed by different capital letters are significantly different between residue treatments (R+ = residues returned, R− = residues removed, U = undisturbed), and means followed by different lower case letters are significantly different between N rates (0 and 160 kg N ha−1), according to a post-hoc Tukey’s mean comparison (p < 0.05). In May 2011, yields of PG biomass were significantly higher in MS than in SG plots; correspondingly the amount of residue returned in R+ subplots was significantly higher in MS than in SG plots. Over the growing season, total (16S rRNA; log10 7.9 – log10 11.3 gene copies g−1 soil) and denitrifying (nirS; log10 4.8 – log10 7.0 gene copies g−1 soil; nosZ; log10 4.2 – log10 6.5 gene copies g−1 soil) gene abundances followed similar patterns (Fig. 1 d, e and f). From May to June sampling dates, denitrifying nirS (log10 2.7 – log10 4.1 transcripts g−1 soil, p < 0.10) and nosZ (log10 1.8 – log10 3.8 transcripts g−1 soil) transcript levels increased in MS plots, while only nirS (not nosZ) transcripts significantly increased in soils from SG plots (p < 0.10), and 16S transcripts levels (log10 7.1 – log10 11.5 transcripts g−1 soil, p < 0.10) decreased significantly in both MS and SG plots (Fig. 1 a, b and c; Table 1). Over the growing season, MS plots supported a significantly larger N2O-reducing (nosZ) community than SG plots, however nosZ gene expression was at similar levels between MS and SG soils (Table 1). N fertilization increased nirS and nosZ gene expression, and residue return increased nosZ gene expression regardless of N rate or PG crop species (Table 1); additionally, the potential activity (cDNA) of the total and denitrifying communities differed between PG crop species over time (significant crop × time interaction) (Table 1, Fig. 1a, b and c).

4. Discussion

Seasonal changes in labile C and N, temperature and soil moisture are often reflected in changes in soil microbial communities (Butterbach-Bahl et al., 2013; Boyer et al., 2006; Bremer et al., 2007; Dandie et al., 2008; Németh et al., 2014; Rasche et al., 2011; Wolings and Priémé, 2004). In previous studies, successional changes in microbial populations during residue decomposition have been observed, with crop residue quality affecting the functioning, functional diversity and community structure of soil microbial communities (Baumann et al., 2009; Bending, 2002). A previous study in an annual (corn) system at our study site found that field-scale N2O fluxes and nosZ transcript levels were inversely correlated in corn plots with residue returned to soils (Németh et al., 2014), suggesting the return of residues to soils could promote reduction of N2O to N2 in situ. Further evidence of the positive effect of residue return on N2O emissions was provided by Congreves et al. (2017), who found that residue return decreased N2O emissions over a 5-year period in an annual rotation. Given that nosZ gene expression was significantly higher in plots with residues returned compared to plots with residues removed regardless of PG crop, it can be hypothesized that the return of residues may favour complete step-wise denitrification and result in lower potential N2O emissions (Congreves et al., 2017). Conversely, Kravchenko et al. (2017) proposed water absorption by soil-incorporated plant residues as a mechanism of microscale N2O emissions, finding higher N2O emissions when residues were incorporated into soils, which has also been observed after residue plow-in (Mutegi et al., 2010). However, in the present study plant residues were surface-applied as incorporation of residues (ie. through tillage) was not possible in these PG systems, indicating that management of residues in PG vs. annual cropping system may have strikingly different impacts on the N2O balance. In a previous study, ploughing a perennial cropping system resulted in a 10-fold increase in soil N2O emissions (Thompson et al., 2016a). In contrast, soils with residue cover are normally cooler during the day and warmer during the night compared to unmulched soils and have increased air permeability and soil water holding capacity (Blanco-Canqui and Lal, 2009), which likely related to differences in microbial community structure (Casack et al., 2011) and selected for different dominant taxa (Stone et al., 2015). Currently, MS produced large yields and likely transpired late into
the growing season (Dohleman and Long, 2009), which was reflected in low gravimetric soil moisture at October sampling. A parallel study conducted over 2 years within the same field trial, which did not include residue manipulations, identified MS as producing larger yields while supporting larger nosZ denitrifying communities than corn-soybean rotations in the 0–15 cm depth (Thompson et al., 2016b), indicating that crops with increased biomass, and therefore increased residue return via leaf loss, may promote N\textsubscript{2}O reduction potential in surface soils. Similarly, we found a larger N\textsubscript{2}O-reducing community present in MS vs. SG soils; however, nosZ expression varied over the course of sampling between PG plots, indicating in the shallow sampling depth assessed in this study, potential N\textsubscript{2}O-reducing activity was not strongly influenced differently by PG crop species.

All changes in community sizes followed similar trends over time, indicating that the proportion of the soil bacterial community with the ability to denitrify remained stable relative to the total bacterial community size. Soil moisture varied over the course of the growing season, however did not vary based on residue manipulations likely due to residues being surface-applied. Precipitation events prior to sampling dates may have increased microbial consumption of alternate electron acceptors such as NO\textsubscript{3}\textsuperscript{-} due to decreased O\textsubscript{2} availability, which also likely influenced soil microbial community size (Németh et al., 2014; Rasche et al., 2011). Soil NH\textsubscript{4}\textsuperscript{+} increased from May to June in SG plots, reflective of other studies which have observed that lowland varieties of SG, as studied here have low N removal rates (Oliveira et al., 2017). A similar significant increase in soil NH\subscript{4}-N and soil moisture was not observed in MS soils from May to October. Additionally, soil NO\textsubscript{3}-N decreased from May to October sampling in fertilized MS plots, indicating a varied influence of PG species on soil N-cycling. A positive response of plant tissue %N to N fertilization in MS (Supp. Table 1) with no corresponding increase in biomass may be indicative of luxury N uptake, which may have decreased microbial access to soil NO\textsubscript{3}-N, and increased selective pressures favouring soil bacteria with the capacity for N\textsubscript{2}O reduction. N fertilization of PGs did not increase gene abundances, supporting results observed in a longer-term parallel study, which indicated no effect of N fertilization on denitrifier community sizes (Thompson et al., 2016b). In contrast, long-term studies with approximately 25–50 years of fertilization history have reported differences in the size and relative proportions of denitrifying communities based on fertilization regimes (Hai et al., 2009; Hallin et al., 2009). Presently, an increase in both nir\textsubscript{S} and nosZ denitrifying transcripts after N application (160 kg N ha\textsuperscript{-1}) was detected, indicating the short-term availability of soil N affected the potential activity of these denitrifying microbial communities, but did not change the size of these communities over the growing season.

The denitrification genes targeted in this study have been found in a variety of bacterial genera and represent a diverse assemblage of microbes with a wide range of functional diversity (Philippot, 2006). The size of these functionally diverse bacterial communities may be controlled distally by edaphic influences, such as water and resource availability while the proximal influences of PG-specific crop inputs, or management (e.g. residue return, N application) were better reflected in differences in denitrifying gene expression. Over just one growing season, we observed that denitrifying transcripts were elevated in N-fertilized PG plots, indicating applying fertilizer in these systems may lead to increased potential denitrifying activity and associated N losses with no return in yield. Residues returned to the soil surface resulted in significantly higher nosZ expression than in soils with surface residues removed, indicating that repeated annual removal of aboveground biomass should not be conducted indiscriminately as it may result in a shift in soil microbial community functioning and associated soil health, and potentially influence the N\textsubscript{2}O balance in PG bioenergy feedstock systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.apsoil.2018.06.005.

References

soilbio.2008.06.024.
Morales, S.E., Cosart, T., Holben, W.E., 2010. Bacterial gene abundances as indicators of
greenhouse gas emission in soils. ISME J. 4, 799–808. http://dx.doi.org/10.1038/
ismej.2010.8.
Muhammad, W., Vaughan, S.M., Dalal, R.C., Menzies, N.W., 2010. Crop residues and
fertilizer nitrogen influence residue decomposition and nitrous oxide emission from a
oxide emissions and controls as influenced by tillage and crop residue management
2010.06.004.
in nitrifier and denitrifier communities associated with a field scale spring thaw N2O
007.
Transition to second generation cellulosic biofuel production systems reveals limited
negative impacts on the soil microbial community structure. Appl. Soil Ecol. 95,
Philippot, L., Martin-laurent, F., Germon, J.C., 2002. Molecular analysis of the nitrate-
reducing community from unplanted and maize-planted soils. Appl. Environ.
Philippot, L., Martin-laurent, F., Germon, J.C., 2002. Molecular analysis of the nitrate-
reducing community from unplanted and maize-planted soils. Appl. Environ.
Philippot, L., 2006. Use of functional genes to quantify denitrifiers in the environment.
Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S.,
Richter, A., Sesstisch, A., 2011. Seasonality and resource availability control bacterial
and archaeal communities in soils of a temperate beech forest. ISME J. 5, 389–402.
http://dx.doi.org/10.1038/ismej.2010.138.
Sokhansanj, S., Mani, S., Turhollow, A., Kumar, A., Bransby, D., Laiser, L.L., Laiser, M.,
2009. Large-scale production, harvest and logistics of switchgrass (Panicum virgatum
L.) – current technology and envisioning a mature technology. Biofuels Bioprod.
Bioprocess. 124–141 https://doi.org/10.1002/bbb.
Stone, M.M., Kan, J., Plante, A.F., 2015. Parent material and vegetation influence bac-
terial community structure and nitrogen functional genes along deep tropical soil
http://dx.doi.org/10.1016/j.soilbio.2014.10.019.
that predict environmental impacts of land use-change for perennial energy crops on
water, carbon and nitrogen cycling. GCB Bioenergy 5, 227–242. http://dx.doi.org/
Thompson, K.A., Bent, E., Abalos, D., Wagner-Riddle, C., Dunfield, K.E., 2016a. Soil
microbial communities as potential regulators of in situ N2O fluxes in annual and
1016/j.soilbio.2016.08.030.
Thompson, K.A., Deen, B., Dunfield, K.E., 2016b. Soil denitrifier community size changes
with land use change to perennial bioenergy cropping systems. Soil. 2, 523–535.
http://dx.doi.org/10.5194/soil-2-523-2016.
Wolsing, M., Priemé, A., 2004. Observation of high seasonal variation in community
structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle
manure by determining T-RFLP of nir gene fragments. FEMS Microbiol. Ecol. 48,
between nitrogen transformation rates and gene abundance in a riparian buffer soil.
Thompson, K.A., Bent, E., Abalos, D., Wagner-Riddle, C., Dunfield, K.E., 2016a. Soil
microbial communities as potential regulators of in situ N2O fluxes in annual and
1016/j.soilbio.2016.08.030.
Thompson, K.A., Deen, B., Dunfield, K.E., 2016b. Soil denitrifier community size changes
with land use change to perennial bioenergy cropping systems. Soil. 2, 523–535.
http://dx.doi.org/10.5194/soil-2-523-2016.
Wolsing, M., Priemé, A., 2004. Observation of high seasonal variation in community
structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle
manure by determining T-RFLP of nir gene fragments. FEMS Microbiol. Ecol. 48,