REGULAR ARTICLE

Nitrogen transformations revealed by isotope dilution in an organically fertilized hybrid poplar plantation

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Abstract Measuring nitrogen (N) transformations from organic fertilizers can help in selecting applications rates that provide sufficient soluble N to promote tree growth in short-rotation plantations. The objective of this study was to determine how organic fertilizers (papermill biosolids, liquid pig slurry) affected microbially-mediated N transformations in soils. Soil samples were collected from a hybrid poplar plantation before fertilization, 1 month after fertilizer application and at the end of the growing season. Net N mineralization and nitrification were evaluated during a 28 d laboratory incubation, while gross N transformations were assessed using a ^{15}N isotope dilution technique. Pig slurry application increased soil ammonium (NH$_4$-N) and nitrate (NO$_3$-N) concentrations within 1 month, while papermill biosolids increased soil NH$_4$-N and NO$_3$-N concentrations at the end of the growing season. Gross N consumption rates were greater than gross N production rates. The NH$_4$-N and NO$_3$-N consumption rates were positively correlated with labile carbon and microbial biomass. The gross nitrification rate was 18 to 67% of the gross mineralization rate but 30% or less of the gross NH$_4$-N consumption rate, indicating that NH$_4$ consumption was overestimated by the isotope dilution technique. We conclude that N cycling in this hybrid poplar plantation was characterized by rapid consumption of plant-available N following N mineralization and nitrification.

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Abbreviations

CTRL	unfertilized control
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
HSD	honestly significantly different
IF	inorganic fertilizer
LPS	liquid pig slurry
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
PB	papermill biosolids

Introduction

Understanding the pattern of N release from fertilizers, relative to plant growth, is essential to maximize N use efficiency and prevent unnecessary loss of N to the environment. This is particularly challenging when organic fertilizers are applied because the N release from such materials depends on the microbially-mediated processes of N mineralization and nitrification, which are influenced by environmental conditions (temperature, moisture), soil properties (pH, texture, organic matter content) and organic fertilizer characteristics like the C:N ratio, lignin content and particle size (Myrold 1998; Van Kessel and Reeves 2002; Stadler et al. 2006). Measuring the net change in NH_4-N and NO_3-N concentrations is the most common method of assessing N transformations but does not account for the inorganic N immobilized in microbial biomass or lost through denitrification during soil incubation (Hart et al. 1994). Mineralization of organic fertilizers releases inorganic N, but consumption processes that deplete the inorganic N pool are likely when soils are amended with organic fertilizers containing carbon (C)-rich substrates that stimulate microbial activity and growth (Burger and Jackson 2003; Habteselassie et al. 2006). The simultaneous production and consumption of inorganic N from organic fertilizers impacts N availability to plants and can only be resolved by measuring gross N transformations with an isotope dilution technique (Stark 2000).

Two organic fertilizer sources with contrasting characteristics that could affect N transformations are papermill biosolids (PB) and liquid pig slurry (LPS). A byproduct of the papermaking industry, PB presents a range of chemical and microbial characteristics depending on the fiber source, chemical and mechanical processing at the papermill and secondary treatments like composting (Camberato et al. 1997; Feldkirchner et al. 2003), but it is generally a lignin-rich material containing less than 1% NH_4-N (Chabot et al. 2000). Non-composted papermill sludge (C:N ratio=95) caused N immobilization in soil for 16 wks (Burgos et al. 2006), whereas N immobilization was observed for 36 wks in a soil amended with non-composted PB having a C:N ratio of 480 (Zibilske 1987). Land application of composted PB having C:N ratios of 14 to 42 reduced the N immobilization period to about 6 wks (Zibilske 1997; Camberato et al. 2006). We are not aware of reports on the gross N

transformations with PB, but it is expected that gross N consumption will exceed gross N production for weeks to months after N-poor PB is applied.

A N-rich organic fertilizer, LPS contains 50% or more soluble NH_4-N at the time of application and the organic N in LPS is readily mineralized during the growing season (Sommer and Husted 1995). Rapid N mineralization in soils amended with LPS is related to its low C:N ratio (generally less than 4; Sánchez and González 2005) and releases NH_4-N that may be absorbed by plants or undergo other transformations, including NH_3 volatilization, NH_4-N fixation in clays, N immobilization, nitrification and denitrification (Sieling et al. 1998; Rochette et al. 2001; Chantigny et al. 2004). Several studies indicate that soils amended with LPS have nearly equal rates of NH_4 production and consumption (Morvan et al. 1996; Dambreville et al. 2006; Luxhøi et al. 2007). Müller et al. (2003) reported that gross nitrification rates were 20 times greater in grassland soils amended with liquid cattle slurry than the unfertilized control due to the availability of C substrates for heterotrophic nitrifiers, which could be bacteria or fungi (Hayatsu et al. 2008; Laughlin et al. 2008). This may also be the case for LPS, but needs to be confirmed.

Information on N transformations from PB and LPS is available for cultivated agricultural soils, but little is known about the N transformations of these organic fertilizers in short rotation forestry. Plantations of fast-growing woody species such as hybrid poplar (*Populus* spp.) are being established throughout North America in response to demands for timber, pulp and paper and biomass energy (Mead 2005). In eastern Canada, most of the short rotation hybrid poplar plantations were established on marginal or degraded agricultural lands that are not inherently productive due to low soil fertility. Hybrid poplar growth improves when N is added from inorganic fertilizers (Brown and van den Driessche 2002; Cooke et al. 2005) and organic fertilizers (Lteif et al. 2007). However, foliar analysis revealed below-optimum N concentrations, even when trees received an estimated 130 to 140 kg N ha^{-1} y^{-1} of plant-available N from organic fertilizers (PB, LPS and a combination of the two sources) (Lteif et al. 2008). This may indicate that inorganic N consumption exceeded production in this plantation, resulting in low concentrations of plant-available N. Measuring gross N transformations from organic fertilizers is

necessary to verify that application rates of organic fertilizers provide sufficient soluble N to promote tree growth and foliar nutrition.

The objective of this study was to determine the N transformations of PB- and LPS-amended soils in a hybrid poplar plantation by 1) evaluating net N mineralization and nitrification rates and 2) character-izing gross N mineralization, nitrification and con-sumption rates using a short term ^{15}N isotopic pool dilution technique. The hypotheses were that inorganic N consumption exceeded production in the hybrid poplar plantation, and that PB and LPS amendments affected N transformations through their direct effects on labile C substrates and soil microbial biomass.

Materials and methods

Experimental site and fertilizer treatments

Soils in this study were collected from a hybrid poplar plantation in St. Camille, Québec, Canada (45°40′36″N, 71°44′13″W). The plantation was established in 2001 on an unimproved hayfield with low soil fertility and other production constraints (poor drainage, stones in the mineral layer and substratum). Surface soil (0-15 cm depth) was fine loamy, mixed, cool Typic Humaquept (Orthic Gleysol in the Canadian classification system, Soil Classifica-tion Working Group 1998) of the Magog series. This clayey loam soil contained 390 g sand kg^{-1}, 320 g silt kg^{-1} and 290 g clay kg^{-1}, with 30 g organic C kg^{-1} and pH $_{water}$ of 5.6 when hybrid poplars were planted.

Details of the experimental design, treatments and fertilizer sources were reported by Lteif et al. (2007). Briefly, the experiment was a randomized complete block design with 10 fertilizer treatments, replicated in 4 blocks, for a total of 40 experimental plots. Each plot (9 m^2) had 16 trees of *Populus trichocarpa x Populus deltoides*, clone 3225, with 3 m spacing between tree rows. Fertilizer treatments were applied during the fourth year (2004) and fifth year (2005) after plantation establishment. This study focused on nitrogen transformations in soils from 6 treatments during the 2005 growing season. There were unfer-tilized controls (CTRL) and inorganic fertilizer (IF) applied at 35 kg N ha^{-1}. The N input from organic fertilizers was converted to an inorganic N fertilizer equivalent value, as described by Lteif et al. (2008)

and was estimated to be 65 kg N ha^{-1} (PB65) and 130 kg N ha^{-1} (PB130) for PB-amended soils and 70 kg N ha^{-1} (LPS70) and 140 kg N ha^{-1} (LPS140) for the LPS-amended soils. Fertilizers were spread evenly in a circle (< 1 m radius) around the trunk of four trees per plot in mid-May, and left on the soil surface (unincorporated). Fertilizer sources in the IF-amended soils were calcium ammonium nitrate (35 kg N ha^{-1}) and triple superphosphate (30 kg P$_2$O$_5$ ha^{-1}). The PB65 application consisted of about 3.9 kg m^{-2} of biosolids (wet weight, ww) and about 2.1 kg m^{-2} of pig slurry (ww) was applied to the LPS70-amended soils. These application rates were doubled in the PB130- and LPS140-amended soils. Biosolids came from a papermill owned by Domtar Inc. (Windsor, Quebec, Canada) and contained an average of 26 g total N kg^{-1} dry weight (dw), 78 kg H$_2$O kg^{-1} and a C/N ratio of 16, while the pig slurry was from a piggery in St. Camille (Quebec, Canada) and contained an average of 462 g total N kg^{-1} dw, 99 kg H$_2$O kg^{-1} and had a C/N ratio of 0.7 (Lteif et al. 2007).

Soil sampling

Soil samples were collected at three sampling times: before fertilizer application in May 2005 (PreF), about 1 month after fertilizer application in June 2005 (PostF) and before leaf senescence in October 2005 (EndF). Within each replicate plot (4 replicates per treatment=24 plots in total), a composite soil sample was created by mixing subsamples (about 500 g each) collected with a shovel from the 0-15 cm depth within 1 m of each fertilized tree. Weeds and surface litter around the tree were removed by hand before soil was collected. Soil samples were coarsely sieved (<6 mm) in the field to remove rocks and large residues, placed in sealed polyethylene bags and transported to the laboratory on ice to minimize microbial activity. Additional sieving (<4 mm mesh) was done in the laboratory to remove fine residues and roots, and samples were stored at 4°C until analysis.

Soil analyses

Microbial biomass C and N (MBC, MBN) concen-trations in field-moist soil samples from each plot were determined using the chloroform fumigation-direct extraction method described by Vance et al.

(1987). Paired subsamples were either directly extracted with $0.5M$ K_2SO_4 (1:4 soil:extractant) or fumigated with chloroform daily for 5 d before extraction. The dissolved organic C (DOC) concentration in unfumigated and fumigated soil extracts was determined using a Shimadzu TOC-V analyzer (Shimadzu Corporation, Kyoto, Japan). The MBC was calculated as [(DOC in fumigated soil extracts – DOC in unfumigated soil extracts)/k_{EC}] where k_{EC} is the extraction coefficient 0.45 (Wu et al. 1990). For microbial biomass N (MBN), the fumigated and unfumigated extracts underwent persulfate digestion (Cabrera and Beare 1993) and colorimetric analysis for NH_4-N and NO_3-N using a Lachat Quik Chem AE flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). The MBN was calculated as [(total N in digests of fumigated soil extracts – total N in digests of unfumigated soil extracts)/k_{EN}] where k_{EN} is the extraction coefficient 0.54 (Brookes et al. 1985). The dissolved organic N (DON) concentration was [total N in digests of unfumigated soil extracts – (NH_4-N + NO_3-N in unfumigated soil extracts)].

Net N mineralization and nitrification

About 25 g of field-moist soil was packed in a 120-ml polyethylene cup to a bulk density of 0.61 g cm^{-3} and the moisture content was adjusted to 60% water-filled pore space, assuming a particle density of 2.65 g cm^{-3} (Elliott et al. 1999). Cups were placed in 1 L glass Mason jars, with 10 ml of water in the bottom of each jar to maintain soil humidity, sealed with an air-tight lid and incubated at 21°C in the dark. Every 7 d, the jars were aerated for 15 min. After 28 d, mineral N (NH_4-N and NO_3-N) concentrations in 0.5 M K_2SO_4 extracts (1:2 soil:extractant) of the incubated soil, as well as non-incubated soil, were determined with the Lachat flow injection autoanalyzer. The N mineralization rate (mg NH_4-N kg^{-1} d^{-1}) was the difference in NH_4-N concentrations of incubated and non-incubated soils, divided by the incubation time (28 d), and the nitrification rate (mg NO_3-N kg^{-1} d^{-1}) was calculated in a similar manner.

Nitrogen transformations assessed by ^{15}N isotope pool dilution and enrichment

Gross N mineralization and gross nitrification rates were estimated from the change in size and isotopic composition of soil inorganic N pools ($^{14+15}$ NH_4-N and $^{14+15}$ NO_3-N pools) using disturbed soil cores in the lab (Hart et al. 1994; Cookson et al. 2002). Four containers containing field-moist soil were prepared for each sample collected at the three sampling times (24 samples x 3 times x 4 containers=288 containers). We placed 25 g of field-moist soil in a 120-ml polyethylene cup at a bulk density of 0.61 g cm^{-3} for rapid extraction of mineral N and ^{15}N within 15 min of adding the ^{15}N solution (t_0). Two polyethylene cups were prepared for each soil sample. This container and soil mass were chosen to eliminate the need for further handling and thus provide an unbiased estimate of soil N pools at t_0. We packed 150 g of field-moist soil into a 10 cm x 5 cm aluminum cylinder at a bulk density of 0.61 g cm^{-3} for assessment of the microbially-mediated transformations of mineral N and ^{15}N after 48 h (t_{48}). Two cylinders were prepared for each soil sample. A larger soil mass was selected for these samples to better represent the soil microbial community involved in N transformations during a 48 h period.

Solutions containing total N and ^{15}N were added to soil cores to increase the NH_4-N and NO_3-N pools and provide sufficient ^{15}N to detect isotope dilution and enrichment. In most soils, the initial NH_4-N concentration was less than 20 mg kg^{-1}, while the initial NO_3-N concentration was less than 10 mg kg^{-1}; the solutions added increased the NH_4-N concentration by 2.5 fold, on average, and the NO_3-N concentration by 1.5 times. For gross N mineralization, soil in one polyethylene cup (t_0) and in one aluminum cylinder (t_{48}) received 25 mg $^{14+15}$ N kg^{-1} soil from a solution containing 5.016 atom% ^{15}N from ($^{15}NH_4$)$_2SO_4$. For the gross nitrification study, the other t_0 and t_{48} containers received 5 mg $^{14+15}$ N kg^{-1} soil of a solution containing 5.016 atom% ^{15}N from K$^{15}NO_3$, but soils with a NO_3-N concentration greater than 10 mg $^{14+15}NO_3$-N kg^{-1} received 50 mg $^{14+15}$ N kg^{-1} containing 10.042 atom% ^{15}N from K$^{15}NO_3$.

The ^{15}N -labeled solutions were added to the soil using an automatic pipette (25 g of soil for t_0 samples) or injected uniformly using a 60-mm side-port spinal-needle (150 g of soil for t_{48} samples) as suggested to avoid preferential consumption of ^{14}N versus ^{15}N in aluminum cylinders during the 48 h period (Davidson et al. 1992; Herrmann et al. 2005). Deionized water was added, if necessary, to adjust the soil moisture to 60% water-filled pore space. The t_0 samples were extracted with 0.5 M K_2SO_4

solution (1:4 soil:extractant) within 15 min of ^{15}N addition, while aluminum cylinders containing 150 g of soil were placed in 1 L Mason jars and aerobically incubated in the dark at 21°C for 48 hrs. Then, soil was removed from the cylinder, homogenized and a 25 g subsample was extracted with 0.5 M K$_2$SO$_4$ solution (1:4 soil:extractant). The K$_2$SO$_4$ solution was chosen as a multi-purpose extractant that can be used to measure NH$_4$-N and NO$_3$-N concentrations as well as dissolved organic and microbial biomass C and N concentrations. Soil extracts were frozen until analyzed for mineral N concentrations and atom % ^{15}N enrichment.

The ^{15}NH$_4$-N and ^{15}NO$_3$-N enrichment in soil extracts was determined using a modification of the acid diffusion procedure described by Brooks et al. (1989). The procedure led to the recovery of more than 95% of the ^{15}NH$_4$-N and ^{15}NO$_3$-N from standard solutions prepared and analysed in the same manner as soil extracts. Experimental error due to contamination of reagents and the background ^{15}N concentration was verified by carrying blanks (K$_2$SO$_4$ solution that was extracted and handled in an identical manner as soil extracts) through the experiment. Briefly, the experimental procedure involved diffusing $^{14+15}$NH$_4$-N and $^{14+15}$NO$_3$-N from soil extracts onto acidified filter disks in 120-ml acid-washed polyethylene cups (capped). Soil extracts were spiked with NH$_4$-N solution containing 1.0016 atom % ^{15}N to ensure good recovery of the ^{15}N tracer because significant isotope discrimination occurs during the diffusion step when solutions contain less than 300 μg $^{14+15}$NH$_4$-N (Bradley and Fyles 1996). Although experimental error is introduced when ^{15}N is included in the spiking solution, it provides a means of checking the ^{15}N recovery in each soil extract and thus gives more reliable data (Whalen et al. 2001). The diffusion of ^{15}NH$_4$-N in soil extracts was achieved by adding MgO to the cup, while ^{15}NO$_3$-N diffusion required reaction with Devarda's alloy and MgO. After 7 d, the filter disks were removed, dried in a desiccator over concentrated H$_2$SO$_4$, packed into tin capsules and analyzed for total N and atom % ^{15}N using a PDZ Europa elemental analyzer coupled with a 20-20 isotope ratio mass spectrometer (University of California at Davis Stable Isotope Facility). Gross N mineralization, nitrification, NH$_4^+$ and NO$_3^+$ consumption rates were estimated from the ^{15}NH$_4$ and ^{15}NO$_3$-pool

dilution in the ^{15}N labeled samples (t$_0$ and t$_{48}$) using the equations of Kirkham and Bartholomew (1954). We checked for the possibility of remineralization during the 48 h soil incubation period. Diffused soil extracts ($n=72$) from ^{15}NO$_3$-N amended soils contained 0.946±0.009 atom % ^{15}N in the NH$_4$-N pool due to the NH$_4$-N spiking solution with 1.0016 atom % ^{15}N, so there was no evidence of remineralization, consistent with the assumptions of Kirkham and Bartholomew (1954).

Statistical analysis

The data was tested for normality using the Kolmogorov-Smirnov test and log transformed when necessary to achieve a normal distribution. The effect of fertilizer treatments on extractable C and N pools, microbial biomass, N mineralization and nitrification rates was evaluated for each sampling date using a one-way analysis of variance with SAS statistical software (SAS System 9.1, SAS Institute Inc., Cary, NC). When the treatment effect was significant ($P<$ 0.05), fertilizer treatments were compared with a Tukey multiple comparisons test at the 95% confidence level. Values in tables and graphs are untransformed means± standard errors ($n=4$).

Results

Extractable C and N pools, and microbial biomass

Before fertilizers were applied in spring 2005, plots that were amended with PB140 1 year earlier had a greater NH$_4$-N concentration than the CTRL, but there were no other differences that could be attributed to residual N from fertilizer applications in the previous growing season (Table 1). There was more NH$_4$-N in the LPS 140-amended soil than the CTRL 1 month after fertilization and more NH$_4$-N in the PB65- and PB130-amended soils than in other soils the end of the growing season (Table 1). The NO$_3$-N concentration was greater in most organically fertilized soils than the CTRL at PostF and EndF sampling dates (Table 1). Although the DON concentration was not affected by fertilization, there was more DOC, MBN and MBC in the PB65- and PB130-amended soils than the IF and CTRL soils at the end of the growing season (Table 1).

Table 1 Extractable nitrogen, dissolved organic nitrogen and carbon (DON, DOC) and microbial biomass nitrogen and carbon (MBN, MBC) concentrations in soils (0-15 cm) from a hybrid poplar plantation. Soils were collected shortly before fertilizer application (PreF), about 1 month after fertilizer application (PostF) and just before leaf senescence (EndF). Within a sampling date, mean values±standard errors (n=4) in a column with different letters were significantly different (P<0.05, Tukey's HSD test)

Fertilizer amendment	NH$_4$-N	NO$_3$-N	DON[1]	DOC[1]	MBN[1]	MBC[1]
	(mg kg^{-1})					
PreF						
CTRL	7.7±2.3^{b}	2.6±1.7^{a}	–	–	–	–
IF	8.8±1.^{ab}	1.3±0.5^{a}	–	–	–	–
PB65	14±1.5^{ab}	5.7±1.5^{a}	–	–	–	–
PB130	19±4.6^{a}	2.7±0.3^{a}	–	–	–	–
LPS70	9.6±2.2^{ab}	1.5±0.3^{a}	–	–	–	–
LPS140	9.0±0.7^{ab}	3.0±1.0^{a}	–	–	–	–
PostF						
CTRL	2.7±0.5^{b}	0.9±0.3^{c}	7.0±1.7^{a}	98±22^{a}	138±9.0^{a}	817±23^{a}
IF	4.0±0.4^{b}	2.9±1.1^{bc}	14±2.0^{a}	129±11^{a}	148±23^{a}	1,071±139^{a}
PB65	6.3±0.7^{ab}	4.2±1.0^{b}	13±1.3^{a}	119±7.6^{a}	204±5.7^{a}	1,207±110^{a}
PB130	8.8±1.5^{ab}	2.8±1.4^{bc}	15±1.8^{a}	132±22^{a}	212±25^{a}	1,014±75^{a}
LPS70	17±13^{ab}	18±13^{b}	8.1±1.5^{a}	94±5.0^{a}	165±9.9^{a}	931±48^{a}
LPS140	47±22^{a}	106±35^{a}	9.2±6.4^{a}	112±18^{a}	180± ±11^{a}	1,113±319^{a}
EndF						
CTRL	2.1±0.3^{b}	1.1±0.6^{c}	21±2.6^{a}	78±6.3^{c}	144±28^{c}	804±49^{c}
IF	2.6±0.4^{b}	1.5±0.9^{c}	20±3.9^{a}	125±6.4^{b}	184±30^{c}	879±45^{c}
PB65	5.8±0.6^{a}	20±1.3^{ab}	23±4.3^{a}	204±22^{a}	347±55^{ab}	1,561±161^{ab}
PB130	8.4±1.1^{a}	39±6.4^{a}	21±7.1^{a}	274± ±25^{a}	532±54^{a}	2,248±220^{a}
LPS70	2.7±0.6^{b}	4.1±1.9^{bc}	21±1.5^{a}	100±7.4^{bc}	226±29^{bc}	1,156±137^{bc}
LPS140	3.4±0.5^{b}	7.7±0.3^{ab}	20±2.6^{a}	114±12^{bc}	231±24^{bc}	995±121^{bc}

[1] DON, DOC, MBN and MBC concentrations were not analysed in the PreF soil samples

N mineralization and nitrification rates

There was a decline in the soil NH$_4$-N concentration during the 28 d laboratory incubation at all sampling dates, producing net N mineralization rates of -0.1 to -1.6 mg NH$_4$-N kg^{-1} d^{-1} (data not shown). Net nitrification rates were <1 mg NO$_3$-N kg^{-1} d^{-1} at the PreF sampling date and ranged from -2.9 to 1.7 mg NO$_3$-N kg^{-1} d^{-1} at the PostF sampling date, with significantly (P<0.05, Tukey's test) less nitrification in the LPS140-amended soil than other soils (Fig. 1a). The net nitrification rate was greater in the PB65- and PB130-amended soils than other soils at the end of the growing season (Fig. 1a).

Gross N mineralization rates ranged from 0 to 14.6 mg NH$_4$-N kg^{-1} d^{-1}. There was significantly (P< 0.05, Tukey's test) more gross N mineralization in the PB65- and PB130-amended soils than soil receiving LPS140 at the PostF sampling date and in the PB130-amended soil than other soils at the EndF sampling date (Fig. 1b). Gross nitrification rates were between 0.1 and 8.0 mg NO$_3$-N kg^{-1} d^{-1} and were not affected by fertilizer treatments (Fig. 1c).

The NH$_4$-N and NO$_3$-N production rates were less than consumption rates, as gross N mineralization rates were 2 to 43% of the NH$_4$-N consumption rates and gross nitrification rates were 1 to 26% of the NO$_3$-N consumption rate during the study (Fig. 1b, c, Table 2). The ratio of gross nitrification to gross mineralization was not significant, with negligible values on the PreF sampling date and ratios of 0.13 to 0.78 on the PostF and EndF sampling dates. The ratio of gross nitrification to NH$_4$-N consumption rate did not exceed 0.30, with no difference between treatments at any sampling date (Table 2). Except for the net mineralization rate, N transformations at the PostF and EndF sampling dates were positively correlated with DOC, MBN and MBC concentrations (Table 3).

Discussion

Other studies have reported on N transformations in cultivated agricultural soils amended with PB and LPS, but in this study, the organic fertilizers were applied and left on the soil surface (unincorporated) because tillage

Fig. 1 Nitrogen transformation rates in soils (0-15 cm) from a hybrid poplar plantation showing a) net nitrification rate (mg NO_3-N kg^{-1} d^{-1}), b) gross mineralization (mg NH_4-N kg^{-1} d^{-1}) and c) gross nitrification (mg NO_3-N kg^{-1} d^{-1}). Soils were collected just before fertilizer application (PreF), 1 month after fertilizer application (PostF) and just before leaf senescence (EndF). Within a sampling date, bars with the same letter are not statistically different ($P<0.05$, Tukey test)
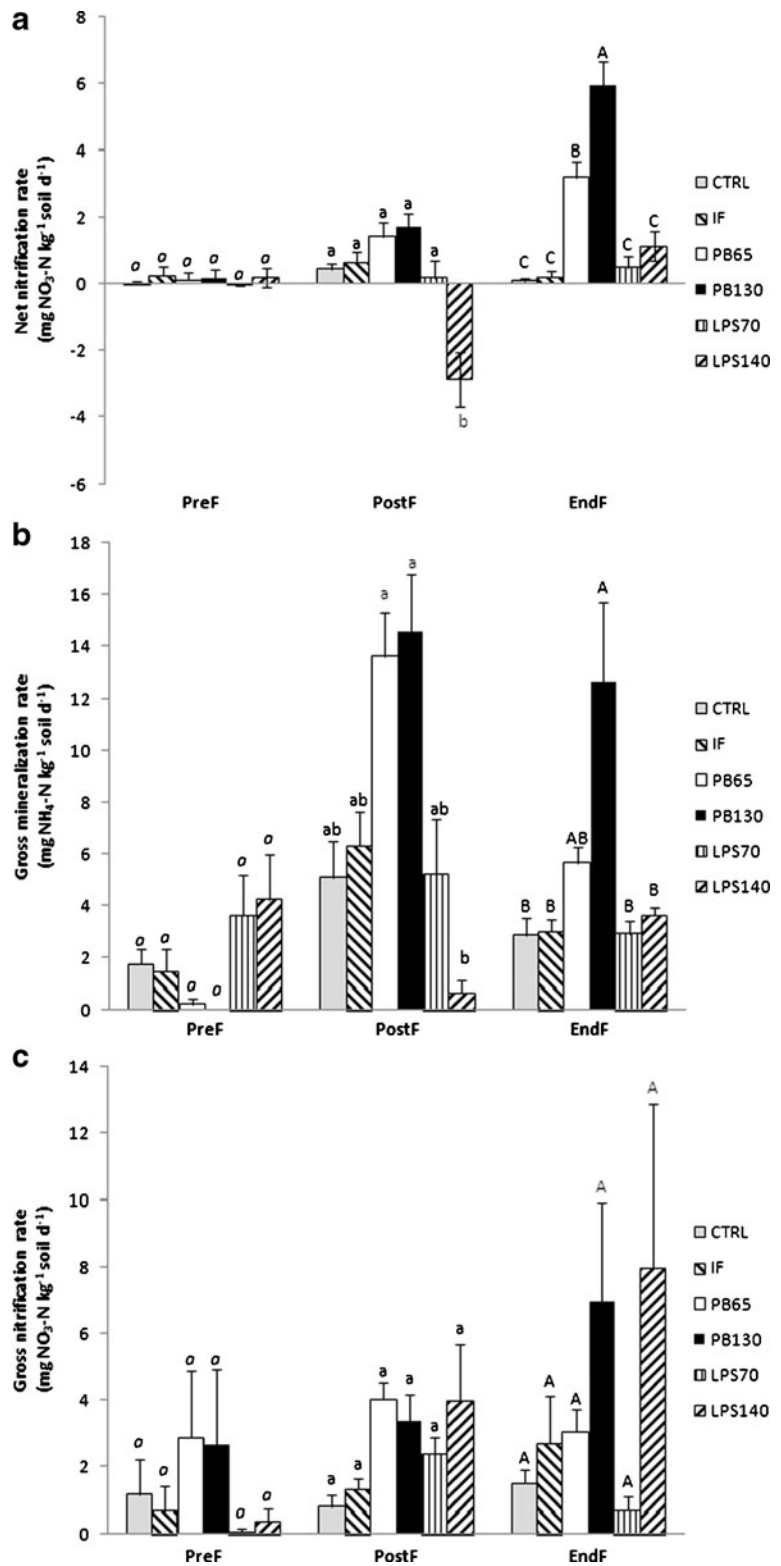
Table 2 The NH_4-N consumption (c_A) and NO_3-N consumption (c_N) rates in soils (0-15 cm) from a hybrid poplar plantation. The ratio of the gross nitrification rate (n) to gross mineralization rate (m) and the n to c_A ratio are also provided. Soils were collected shortly before fertilizer application (PreF), about 1 month after fertilizer application (PostF) and just before leaf senescence (EndF). Within a sampling date, mean values ± standard errors ($n=4$) in a column with different letters were significantly different ($P<0.05$, Tukey's HSD test)

Fertilizer amendment	c_A mg NH_4-N kg^{-1} d^{-1}	c_N mg NO_3-N kg^{-1} d^{-1}	n/m ratio	n/c_A ratio
PreF				
CTRL	17±0.8[a]	14±3.9[a]	0.05±0.05	0.07±0.06
IF	18±0.7[a]	14±1.6[a]	0.56±0.56	0.04±0.03
PB65	16±0.6[a]	23±3.0[a]	ND[a]	0.18±0.13
PB130	16±0.3[a]	23±4.3[a]	ND	0.25±0.17
LPS70	20±1.4[a]	14±2.0[a]	0.01±0.01	0.01±0.01
LPS140	20±2.1a	14±1.4[a]	0.06±0.06	0.02±0.01
PostF				
CTRL	22±1.6[ab]	7.7±1.1[b]	0.13±0.05	0.03±0.01
IF	24±0.8[ab]	9.7±1.3[b]	0.22±0.06	0.06±0.01
PB65	31±1.3[a]	17±2.6[ab]	0.24±0.01	0.10±0.01
PB130	34±2.5[a]	15±0.4[ab]	0.25±0.10	0.10±0.03
LPS70	19±5.4[ab]	14±2.8[ab]	0.45±0.07	0.17±0.05
LPS140	6.1±1.9[b]	29±7.8[a]	ND	0.30±0.20
EndF				
CTRL	20±1.7[b]	8.8±2.3[c]	0.78±0.19	0.08±0.02
IF	22±0.8[b]	11±3.7[c]	0.78±0.29	0.12±0.07
PB65	25±0.2[ab]	50±2.4[b]	0.67±0.19	0.14±0.02
PB130	34±2.7[a]	75±8.6[a]	0.47±0.20	0.19±0.10
LPS70	22±0.8[b]	15±3.4[c]	0.18±0.11	0.03±0.02
LPS140	22±0.4[b]	25±5.7[bc]	ND	0.04±0.02

[a] ND=not determined because n > m

could damage tree roots. The PB gradually decomposed and disappeared from the soil surface during the growing season, probably mixed into mineral soil by earthworms observed in the 0–15 cm layer. Gradual decomposition of PB may account for the higher NH_4-N, NO_3-N, DOC, MBN and MBC concentrations in the PB65- and PB130-amended soils than the CTRL at

Table 3 Pearson correlation coefficients (r) between nitrogen transformation rates and dissolved organic carbon (DOC), microbial biomass nitrogen and carbon (MBN, MBC) concentrations in soils (0-15 cm) from a hybrid poplar plantation. Data were from soils collected about 1 month after fertilizer application and just before leaf senescence ($n=48$)

N transformation rate	DOC	MBN	MBC
Net mineralization	0.001[NS]	0.037[NS]	0.011[NS]
Net nitrification	0.742[***]	0.793[***]	0.653[***]
Gross mineralization	0.454[**]	0.416[**]	0.508[**]
NH_4-N consumption (c_A)	0.484[**]	0.452[**]	0.542[***]
Gross nitrification	0.481[**]	0.496[**]	0.528[***]
NO_3-N consumption (c_N)	0.837[***]	0.897[***]	0.826[***]

Correlation coefficients were significant at $P<0.05$[*], $P<0.01$[**], $P<0.001$[***] or not significant (NS)

the end of the growing season. In contrast, water-soluble nutrients in the LPS probably entered the soil when this organic fertilizer was applied, but LPS left on the soil surface is susceptible to N loss via volatilization (Rochette et al. 2001; Chantigny et al. 2004). Hence the soil NH_4-N and NO_3-N concentrations tended to be greater in the LPS70- and LPS140-amended soils than the CTRL only in the first month after fertilizer application. Organic fertilizer effects on soil NH_4-N and NO_3-N concentrations during the growing season are consistent with the pattern of N release from organic fertilizers predicted in our previous work. Based on foliar analysis, Lteif et al. (2008) suggested that PB application provided a steady source of plant-available N throughout the growing season, whereas LPS supplied plant-available N for a short period, which increased the leaf mass of trees in the LPS-amended soil relative to the unfertilized control but produced foliage with a suboptimal N concentration. Two other methods for evaluating foliar nutrition, the critical value approach and compositional nutrient diagnosis (Ulrich and Hills 1967; Parent et al. 1995), both showed that the N concentration in foliage at the end of the growing season was below the

optimal range, causing nutrient imbalances that were negatively correlated with hybrid poplar growth (Lteif et al. 2008). This suggests that the measured NH_4-N and NO_3-N pool sizes were not a good indication of the N availability to hybrid poplars.

Our 28-d soil incubation study indicated that net N mineralization rates were negative in all treatments and net nitrification rates did not exceed 6 mg NO_3-N kg^{-1} d^{-1}. The negative net nitrification rate in LPS140-amended soils at the PostF sampling date suggests consumption of more than 150 mg N kg^{-1} (initial NH_4-N plus NO_3-N concentration) during soil incubation. Fertilization is expected to lead to a slight increase in N mineralization in short-rotation forest plantations, as net N mineralization rates of 0.08 to 0.15 mg N kg^{-1} d^{-1} were reported in a 7-year old *Populus deltoides* plantation that received 56 to 224 kg N ha^{-1} y^{-1} from inorganic fertilizer (Lee and Jose 2006). However, organic fertilizers supply labile organic C as well as inorganic N, which appears to strongly favour N consumption in soils from this hybrid poplar plantation, but isotope dilution is the only way to quantify N production and consumption processes.

Gross N transformations

There was no difference in the gross N transformations prior to fertilizer application (PreF sampling date), despite the fact that all plots had received the same treatments in the year preceding this study. As there was little difference in the initial NH_4-N and NO_3-N concentrations between treatments at this sampling date, it suggests that residual fertilizer N left at the end of the 2004 growing season was either immobilized into MBN and soil organic N pools or lost to the environment before May 2005.

Gross N mineralization rates at the PostF and EndF sampling dates were in the same range as those reported in agricultural soils amended with organic fertilizers. Liquid dairy slurry applications to a cultivated field under silage corn production led to gross N mineralization rates of 1 to 5 mg N kg^{-1} d^{-1} during a 4-yr study (Habteselassie et al. 2006), 6.1 mg N kg^{-1} d^{-1} in pasture soil (Zaman et al. 1999) and 3.2 mg N kg^{-1} d^{-1} in a grassland soil (Müller et al. 2003). Agricultural soils receiving solid dairy cattle compost had gross N mineralization rates up to 9 mg N kg^{-1} d^{-1} (Habteselassie et al. 2006),

which is still lower than the 12.7 to 14.5 mg N kg^{-1} d^{-1} measured in PB130-amended soil at the PostF and EndF dates. Soils receiving LPS140 exhibited lower gross N mineralization rates than PB65- and PB130-amended soils at the PostF date, suggesting that labile organic N in the LPS had been metabolized by soil microorganisms within 30 days of application. High N mineralization is related to the availability of C substrates like DOC, which was positively correlated with the gross N mineralization rate in this study and in other studies (Dambreville et al. 2006; Habteselassie et al. 2006).

While gross nitrification rates were not affected by organic fertilizer applications, the rates of less than 1 to 8 mg N kg^{-1} d^{-1} in this study are consistent with other reports. Gross nitrification rates varied between 1-2 mg N kg^{-1} d^{-1} with liquid dairy slurry and 1-14 mg N kg^{-1} d^{-1} with dairy cattle compost during a 4-yr study (Habteselassie et al. 2006). Dairy effluent applications led to a gross nitrification rate of 1.4 mg N kg^{-1} d^{-1} in pasture soils (Zaman et al. 1999) and to 10.9 mg N kg^{-1} d^{-1} on grassland soils, which was 20 times more than the unfertilized control (Müller et al. 2003). Our results indicate a positive correlation between gross nitrification rates and soil carbon pools (DOC, MBC), which is consistent with other reports (Müller et al. 2003; Habteselassie et al. 2006).

The N transformations in soils from a hybrid poplar plantation were characterized by gross N consumption rates that far exceeded production rates. The gross N consumption rates are biased by the fact that adding ^{15}N-labeled NH_4-N and NO_3-N solutions increased the soil mineral N concentrations; microorganisms had more inorganic N available than under natural conditions, which can lead to an overestimation of N consumption. However, this method has been used by other researchers, who report gross NH_4-N consumption rates from 0.5 to 1.5 times greater than the gross N mineralization rate in agricultural soils (Morvan et al. 1996; Müller et al. 2003; Dambreville et al. 2006; Habteselassie et al. 2006; Luxhøi et al. 2007). Our results are even more pronounced - within 1 month of fertilizer application, gross NH_4-N consumption rates were 2 to 6 times the gross N mineralization rate and there was a 3- to 8-fold difference in NH_4-N consumption and production rates at the end of the growing season. This suggests a rapid consumption of NH_4-N released from organic

fertilizers in the hybrid poplar plantation. We noted greater NH_4-N consumption rates in the PB-amended soils compared to the LPS140-amended soils at the PostF sampling date and from PB130-amended soil than other treatments at the EndF sampling date, indicating a tendency for greater NH_4-N consumption in soils receiving PB.

In agricultural soils, NH_4-N consumption does not necessarily have a negative impact on plant growth because nitrifiers are strong competitors for NH_4-N. Habteselassie et al. (2006) reported that the gross nitrification rates accounted for 21% to over 100% of the gross NH_4-N consumption rate in soils amended with inorganic and organic fertilizers. Theoretically, gross nitrification rates cannot exceed gross NH_4-N consumption rates, suggesting some difficulty in estimating these rates with the isotope dilution method, as discussed by Habteselassie et al. (2006). In the hybrid poplar plantation, the gross nitrification rate was only 1 to 30% of the gross NH_4-N consumption rate. Yet, the gross nitrification rate was 18 to 67% of the gross N mineralization rate after fertilizer addition, suggesting that a greater proportion of the NH_4 produced was used by nitrifiers. These results emphasize the tendency of the isotope dilution technique to overestimate gross N consumption rates, particularly when an excess of NH_4-N and NO_3-N is supplied in the ^{15}N-labelling solution. An alternative is to use solutions that are more highly enriched with ^{15}N but contain a lower concentration of mineral N.

Gross NO_3-N consumption rates were up to 8 times the gross nitrification rate at 1 month after fertilizer application and as much as 27 times greater than the gross nitrification rate at the end of the growing season. This differs from the low rates of gross NO_3-N consumption (-4.5 to 4 mg N kg^{-1} d^{-1}) reported in agricultural soils by Habteselassie et al. (2006). In this study, gross NH_4-N and NO_3-N consumption rates were positively correlated with the concentrations of labile C (DOC) and microbial biomass (MBC, MBN), which suggests rapid N immobilization in these soils related to microbial biomass and its activity. As the PB-amended soils tended to have greater DOC and microbial biomass, particularly at the EndF sampling date, the application of PB is expected to promote NH_4-N and NO_3-N consumption to a greater extent than LPS application.

We conclude that N cycling in soils of this hybrid poplar plantation is characterized by rapid immobili-

zation of plant-available N following N mineralization and nitrification, particularly when organic fertilizers containing labile organic C like PB are applied. High rates of NH_4-N and NO_3-N consumption can temporarily reduce the amount of plant-available N, which corresponds to our previous finding of foliar N concentration below the optimal level in this hybrid poplar plantation (Lteif et al. 2008). One way to overcome N limitation could be to apply PB and LPS fertilizers together, as LPS would supply NH_4-N and readily mineralizable organic N within the first month while PB would be more slowly mineralized and excess NH_4-N and NO_3-N would be conserved through consumption processes. Lteif et al. (2007) showed that hybrid poplar growth was greater in plots fertilized with a combination of PB and LPS, suggesting that these fertilizer sources were complementary or interacted to improve soil fertility. Adding a water-soluble inorganic N fertilizer to the organically-fertilized trees could enrich the inorganic N pool and provide plant-available N for optimal hybrid poplar production. The quantity of inorganic N fertilizer required to compensate for N consumption in these soils, as well as the economic and environmental implications of inorganic N fertilizer use remains to be determined. While nitrifying bacteria in fertilized soils could assimilate 18 to 67% of the NH_4-N produced, the rest of the NH_4-N was likely immobilized by heterotrophic microorganisms. The gross NO_3-N consumption rates reported here suggest NO_3-N immobilization by heterotrophic organisms and eventual stabilization in the soil organic N pool, or consumption by denitrifiers. The potential for N loss through denitrification from hybrid poplar plantations will be addressed in a future paper.

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