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Nitrous oxide production and potential denitrification in soils from riparian buffer strips: Influence of earthworms and plant litter

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ABSTRACT

Vegetated riparian buffer strips have been established in Southern Quebec (Canada) in order to intercept nutrients such as nitrate (NO_3^-) and protect water quality near agricultural fields. Buffer strips may also favour denitrification through a combination of high soil moisture, NO_3^- and carbon supply, which could lead to the production of nitrous oxide (N_2O), a greenhouse gas. Denitrification could be further amplified by the presence of earthworms, or by plant species that promote earthworm and bacterial activity in soils. Soils from four farms, comprising maize fields and adjacent buffer strips, were sampled in the fall of 2008. A total of six earthworm species were found, but average earthworm biomass did not differ between buffer strips and maize agroecoecosystems. Nitrate concentrations and net nitrification rates were higher in the maize fields than in the buffer strips; there was no difference in N_2O production in soils collected from the two sampling locations. Potential denitrification, measured by acetylene inhibition, varied by two orders of magnitude, depending on experimental conditions; when amended with H_2O or with $H_2O + NO_3^-$, potential denitrification was higher ($P < 0.05$) in soils from buffer strips than from maize fields. Potential denitrification was highest in soils amended with H_2O + glucose, or with $H_2O + NO_3^-$ + glucose. Using microcosms, we tested the effect of litter–soil mixtures on earthworm growth, and the effect of earthworm-litter–soil mixtures on potential denitrification. Based on four categories of chemical assays, litters of woody species (oak, apple, *Rhododendron*) were generally of lower nutritional quality than litter from agronomic species (alfalfa, switchgrass, corn stover). Alfalfa litter had the most positive effect, whereas apple litter had the most negative effect, on earthworm growth. Potential denitrification was 2–4 times higher in earthworm–litter–soil mixtures than in plain soil. Litter treatments that included corn stover had lower potential denitrification than those that included alfalfa or switchgrass, whereas litter treatments that included oak had lower potential denitrification than those that included apple or *Rhododendron*. Results suggest that potential N_2O emissions may be higher in riparian buffer strips than in adjacent maize fields, that N_2O emissions in buffer strips may be amplified by comminuting earthworms, and that plant litters that reduce earthworm growth may not be best at mitigating N_2O emissions.

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1. Introduction

Thousands of kilometres of streams cross the agricultural landscape of Southern Quebec (Canada). Riparian buffer strips, containing perennial grasses and other herbaceous species, have been established along these streams to intercept nutrients in runoff and eroded sediments, thus protecting water quality (Schultz et al., 1997). Of particular concern is nitrate (NO_3^-) pollution, owing to the large quantities of mineral N fertilizers that are applied to agroecosystems each year, the high mobility of this anion in soil solution and its contribution to eutrophication in aquatic ecosystems (Leeds-Harrison et al., 1999). Vegetated riparian buffer strips are intended to be a sink for NO_3^- (i.e., through plant N uptake) as it moves towards the stream.

Denitrification is another process that removes NO_3^- from buffer strips and is considered to be environmentally beneficial when reactive N is fully reduced to N_2 gas. However, the incomplete dissimilatory reduction of NO_3^- by heterotrophic bacteria may lead to N_2O emissions as high as 30% of the reducible soil NO_3^- pool (Nyborg et al., 1997). As N_2O is 300 times more effective as a greenhouse gas (GHG) than CO_2 and contributes to stratospheric ozone depletion, it is an undesirable end product of NO_3^- reduction (Cavigelli and Robertson, 2000). Denitrification is favoured by low oxygen (O_2) concentrations, and by the availability of NO_3^-

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and reduced carbon (Paul and Clark, 1996). It is possible that these three factors occur jointly in riparian buffer strips more so than in adjacent maize fields because buffer strips occur near streams, they lie downslope of fields receiving N fertilizer, and they regularly receive litter and root exudates from perennial vegetation.

Denitrification in riparian buffer strips is also expected to be influenced by earthworm activity. Through their burrowing activities, anecic earthworms may create preferential flow pathways that increase water infiltration and nutrient leaching (Domínguez et al., 2004). For example, Costello and Lamberti (2008) reported an increase in NO_3^- leaching from riparian buffer strips into adjacent aquatic ecosystems due to the burrowing activities of earthworms. Even if some of the NO_3^- produced or transiting through buffer strips can be leached through earthworm burrows, the lining of burrow walls, earthworm casts and even their gut are favourable microenvironments for denitrifiers, possibly contributing to N_2O production in soils (Parkin and Berry, 1994, 1999; Borken et al., 2000; Horn et al., 2003).

It is unclear whether vegetated riparian buffers are a preferred habitat for earthworms. For example, Smith et al. (2008) found higher earthworm biomass in grassy strips at the margin of arable fields in the UK, whereas Lagerlof et al. (2002) found the opposite result in Sweden. Riparian buffer strips in Quebec are required to be at least 1 m wide and usually consist of herbaceous vegetation that may favour earthworm populations (Bohlen et al., 2005). Over recent years, there have been pilot studies in Quebec to increase the width of these buffer strips to as much as 8 m, and to introduce perennial tree and shrub species (Michaud et al., 2005; Fortier et al., 2010). These could, in turn, generate plant litters with a range of chemical characteristics influencing earthworm growth (e.g., Aplet, 1990), and possibly earthworm-mediated denitrification rates. For example, litters of woody plants are expected to be more acidic, more lignified, and contain higher concentrations of phenolic substances, which would make them less palatable and nutritious to earthworms than litter from herbaceous agronomic crops (Aira et al., 2006; Curry and Schmidt, 2007). Of particular interest in the present study is the effect of litter mixtures that would occur at the boundary between agronomic crops such as corn, soybean and switchgrass, and woody species such as oak, apple and ericaceous shrubs that could eventually be used as riparian vegetation in southern Québec.

The three objectives of our study were: (1) to compare soil physico-chemical characteristics and earthworm populations in paired riparian buffer strips and maize fields, (2) to compare N_2O production and potential denitrification in these two sampling locations; and (3) to determine earthworm growth and potential denitrification from plant litter-soil mixtures.

2. Materials and methods

2.1. Earthworm, soil and litter collection

In mid-October 2008, earthworms and soils were sampled in four different maize (*Zea mays* L.) fields, each adjacent to streams and possessing a riparian buffer strip, within a 10 km radius north-east of the Town of Bedford, Québec, Canada (45°07′ N, 72°59′ W). Each stream discharges into *Rivière aux Brochets*, which flows into the Missisquoi Bay at the northern tip of Lake Champlain, New York, USA. The area is exclusively under agricultural production, the average farm being 75–100 ha in size with maize and soybean (*Glycine max* (L.) Merr.) as the two main crops. The four fields were part of a pilot project aimed at increasing buffer strips to 7 m in width. Buffer strips in fields 1 and 2 were planted with switchgrass (*Panicum virgatum* L.) whereas those in fields 3 and 4 were planted with hay mixtures common to Southern Quebec (e.g., timothy (*Phleum pratense* L.), fescue (*Festuca* sp.), clover (*Trifolium* sp.), etc.). In each field, two transects were established parallel to the stream, one in the buffer strip at 3 m from the stream bank, the other at 50 m from the stream within the maize field. Four sampling points were established at 50 m intervals along each transect. At each sampling point, earthworms were collected by excavating one soil pit (0.3 m × 0.3 m × 0.15 m) and hand-sorting to collect specimens. Dilute formalin solution (0.5% formaldehyde) was then poured into the bottom of each pit until saturated, and deeper-dwelling earthworms were collected shortly after the solution had drained through macro-pores. Earthworms from each pit were preserved in 5% formalin solution until the number and biomass of intact individuals and fragments could be assessed in the laboratory. Earthworms with a fully-developed clitellum were identified to the species level using the key of Reynolds (1977). Earthworm biomass was expressed on an ash-free dry weight (AFDW) basis, which is the earthworm dry mass (105 °C for 24 h) minus the soil mass remaining when earthworms were ashed in a muffle furnace (450 °C for 4 h). Around each earthworm sampling pit, four soil subsamples (~500 g) were collected (0–30 cm depth) using hand shovels, passed through a 2 mm sieve and pooled into one composite sample (~2 kg). The 32 composite samples were stored in plastic bags under ice packs, transported to the laboratory and kept at 2 °C until analyses began, one-week later.

During the same week earthworms and soils were sampled, we collected approximately 500 g (dry wt equiv.) of six litter types. Litters of switchgrass, alfalfa (*Medicago sativa* L.) and corn stover (i.e., corn stalks remaining after harvest) were collected from the experimental farm at McGill University's Macdonald Campus (Ste-Anne-de-Bellevue, Québec). Switchgrass is cultivated in southern Québec as a biofuel crop whereas alfalfa and corn are grown as animal feeds. Green tissues were still apparent in the dried alfalfa litter, which is common for a perennial legume cover crop. Woody species used in this study included northern red oak (*Quercus rubra* L.), apple (*Pyrus malus* L.) and various *Rhododendron* spp. The first of these species is a valuable hardwood currently being tested in riparian buffer strips in Québec (Agriculture and Agri-Food Canada, 2008), the second is a commercially valued fruit crop in Québec often found growing along streams, while the third is an ericaceous shrub that is tolerant to moist conditions. Senesced leaves of northern red oak (*Quercus rubra* L.) were collected as they fell from trees in the Town of Sherbrooke, Québec. Freshly fallen apple (*Pyrus malus* L.) leaves were collected from an orchard near the Town of St-Denis de Brompton, Québec. Freshly fallen leaves of various *Rhododendron* spp. were collected from a municipal garden in the Town of Sherbrooke. Each litter type was lightly rinsed under cold water to remove soil particles and other contamination, dried at 35 °C in an air-draft oven, and coarsely ground in a domestic coffee mill.

2.2. Chemical analysis of soil and litter samples

For each field sampling point, a soil subsample was extracted in 1 N KCl solution, and filtrates were analysed colorimetrically for NH_4^+ and NO_3^- on a Technicon Auto-Analyser (Pulse Instrumentations, Saskatoon, Canada) using the Griess-Ilosvay and Nelson methods (Mulvaney, 1996). A second subsample was incubated aerobically at 22 °C for 30 d, and mineralizable N was measured as KCl extractable $NH_4^+ + NO_3^-$ following the incubation. A third soil subsample was dried (65 °C for 48 h) in an air-draft oven, and gravimetric water content was determined by weight loss. The dried soil was then analysed for total C and N using a Vario Macro CN Analyzer (Elementar GmbH, Hanau, Germany). Another oven-dried subsample was mixed with deionized water (1:2) and the pH of the resulting slurry was measured with a standard hydrogen electrode. The remaining soil was combusted at 450 °C and the remaining mineral fraction was used to determine particle size distribution (i.e., textural class) by the Bouyoucos hydrometer technique (Day, 1965).

The quality of each litter type was based on four categories of chemical assays: (i) elemental concentrations, (ii) proximate C fractions, (iii) acidity and acid-buffering capacity, and (iv) phenolic substances. Litters were oven-dried (65 °C) and finely ground in a MM 400 Mixer Mill (Retsch GmbH, Haan, Germany). Subsamples were analysed for total C and N using a Vario Macro CN Analyzer. Another subsample was digested in H_2SO_4/H_2O_2 and analysed for total base cations (Ca, Mg, K and Na) using an AAnalyst-100 atomic absorption spectroscope (Perkin Elmer Corporation, Norwalk, CT). The same digest was analysed for total P by manual colorimetry (absorbance at 828 nm) using Murphy–Riley reagent (Cade-Menun and O'Halloran, 2007). Proximate C-fractions (crude lipids, sugars, cellulose and lignin) were estimated by sequential extractions according to the protocol described by Ryan et al. (1990). Litter pH was measured in water (1:10). Titratable acids, titratable bases and ash bases were determined following the protocol of Howard and Howard (1990). Hydrolyzable tannins were measured by reacting ground litter with methanol and sulfuric acid, and measuring the resulting methyl gallate by reaction with KIO_3 (Hartzfeld et al., 2002). Condensed tannins were analysed colorimetrically after hydrolysis with butanol/HCl, using the proanthocyanidin assay (Gessner and Steiner, 2005). Total phenolics were determined by rehydrating dried acetone-water extracts with distilled water, Folin–Ciocalteu reagent (Sigma), aqueous Na_2CO_3 (20% w/v) and manually reading solution absorbance at 750 nm (Waterman and Mole, 1994).

2.3. N_2O production and potential denitrification

N_2O production from the 32 composite soil samples (4 sites × 2 sampling locations × 4 sampling points) collected from the field experiment was measured under laboratory conditions. Field moist soil subsamples (25 g dry wt equiv.) were transferred into 125 mL plastic sampling containers. Containers were closed with air-tight lids equipped with septa, injected with 10 mL of ambient air and incubated for 24 h at 20 °C. Preliminary tests revealed that N_2O accumulation rate from 30 min to 24 h followed a linear trend. A 5 mL sample of headspace air from each container was then analysed for N_2O concentration using a Varian CP 3800 gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) equipped with an electron capture detector, using He as carrier gas.

Potential denitrification rate in each of the 32 composite soil samples was measured under six different experimental conditions: (i) unamended, (ii) acetylene (C_2H_2)-enriched atmosphere (iii) C_2H_2-enriched atmosphere, amended with water, (iv) C_2H_2-enriched atmosphere, amended with water and glucose, (v) C_2H_2-enriched atmosphere, amended with water and NO_3^-, and (vi) C_2H_2-enriched atmosphere, amended with water, glucose and NO_3^-. Acetylene inhibits microbial N_2O reductase, the terminal enzyme in denitrification, and can thus be used to assess denitrification rates (Yoshinari and Knowles, 1976). For each trial, we transferred 25 g (dry wt equiv.) subsamples of field moist soil into 125 mL plastic sampling containers, applied the amendments, closed the containers with air-tight lids equipped with septa, and incubated these at 20 °C for 24 h. Acetylene enrichment was achieved by adding 10 mL of C_2H_2 to each container, thereby creating a C_2H_2 partial pressure of about 10 kPa. Water amendments consisted of 5 mL additions of deionized H_2O so as to bring the final gravimetric moisture content in each container to 50–60% (i.e., saturation). For the glucose amendments, soil subsamples were first weighed into 500 mL plastic containers and amended with finely ground glucose (1000 μg C g^{-1} soil) applied with talc as 250 mg mixtures (Bradley and Fyles, 1995). This mixture was dispersed through the soil using a kitchen handmixer with one beater, and soils were then transferred to the sampling containers. For the NO_3^- amendments, KNO_3 was dissolved within the deionized water amendments (500 ppm N), such that 5 mL delivered 2.5 mg N into each soil subsample. Following the incubation, a 5 mL sample of headspace air from each container was analysed for N_2O concentration as described above.

2.4. Earthworm biomass and potential denitrification in earthworm–litter–soil mixtures

The trial consisted of a replicated (n = 10) completely randomized block design with 15 litter treatments. The treatments included the six individual litter types as well as nine litter mixtures composed of each agricultural crop litter (i.e., alfalfa, switchgrass and corn stover) mixed (1:1) with litter of each woody plant (oak, apple, *Rhododendron*). Experimental units consisted of 150 mL plastic containers with perforated lids to which we added 100 g of field moist soil and one earthworm (4.54 ± 1.08 g fresh weight = mean ± S.D.). The soil was a homogeneous mixture of what soil remained from all sampling points, and the earthworms were mature individuals of *Lumbricus terrestris* L. purchased from a supplier of fishing bait. One day before the trial began, earthworms were depurated (i.e., their guts emptied) by leaving them for 24 h on wet tissue paper in a covered bucket containing no soil. Earthworms were then individually rinsed with deionised water, gently wiped dry with paper towel, weighed, and transferred into the plastic containers. The soil in each container was then surface amended with 1.0 g (dry wt) of moistened litter, the lids were put into place, and all containers were placed in an incubator set at 15 °C. The mass of the earthworm in each experimental unit was measured once a week for the next five weeks. Earthworm mass for each of these subsequent measurements was converted to "depurated earthworm mass" using an equation derived by Whalen et al. (2000). Immediately following each mass measurement, the earthworms were returned to the experimental units and 1.0 g (dry wt) of moistened litter was added. Following the five week trial, three experimental units from each litter treatment were randomly selected to determine N_2O production in C_2H_2-enriched atmosphere following the removal of earthworms, and with the addition of water, glucose and NO_3^- as described above.

2.5. Statistical analysis

The main effects of sampling location (buffer strip vs. maize field) on earthworm populations and biomass, and on soil physicochemical properties, were tested by factorial ANOVA that treated each field as a block. The effects of sampling location on earthworm populations and biomass were also tested within each field using *t*-tests. The effects of sampling location and experimental condition (i.e., C_2H_2, H_2O, NO_3^- and glucose additions) on N_2O production and potential denitrification were tested by two-way ANOVA, again using the four maize fields as blocks. Differences among treatment means were explored using Tukey's HSD test. In the earthworm–litter–soil mixtures, the effects of litter treatments on earthworm biomass were tested by repeated measures ANOVA, and the effect of selected treatments (i.e., agricultural vs. woody litters) on final earthworm growth was tested by two-way ANOVA followed by Tukey's HSD test. The relative growth rate [RGR = $(\ln \text{mass})_{wk=x} - (\ln \text{mass})_{wk=(x-1)}$] of each earthworm was calculated for each week of the trial. Subsequently, simple and multiple stepwise regression analyses were performed between attributes of leaf litter quality and the mean RGR of each treatment over the five week trial. The effects of earthworm–litter–soil mixtures on potential denitrification were tested by one-way ANOVA, and single degree of freedom orthogonal contrasts were subsequently used

Table 1
Earthworm species, population size and biomass in cultivated maize fields and adjacent uncultivated riparian buffer strips composed of either switchgrass (Fields 1 and 2) or hay (Fields 3 and 4). Values are the mean ± standard errors ($n = 4$).

Field	Earthworm species[a]	Earthworm population (individuals m^{-2})			Earthworm biomass[b] (g AFDW m^{-2})		
		Maize	Buffer strip	*P*-value	Maize	Buffer strip	*P*-value
1	***Aporrectodea turgida*** *A. tuberculata* A. rosea *Lumbricus terrestris*	25 ± 13	25 ± 14	$P = 0.99$	0.83 ± 0.52	0.82 ± 0.52	$P = 0.97$
2	***A. turgida*** ***A. tuberculata*** *L. terrestris*	81 ± 12	44 ± 12	$P = 0.069$	5.51 ± 0.51	2.36 ± 0.52	$P = 0.005$
3	***A. turgida*** *Allolobophora chlorotica* *A. tuberculata* *L. terrestris* *A. rosea*	68 ± 20	138 ± 27	$P = 0.082$	1.38 ± 0.31	3.98 ± 1.15	$P = 0.072$
4	***A. turgida*** ***Allolobophora chlorotica*** *Eiseniella tetraedra* *A. rosea* *L. terrestris*	98 ± 36	98 ± 31	$P = 0.99$	1.33 ± 0.45	1.61 ± 0.50	$P = 0.69$

[a] Species are listed in order of abundance, and those in bold were numerically dominant.
[b] Average earthworm biomass of the four fields: Maize = 2.26 vs. Buffer strip = 2.19 g AFDW m^{-2} ($N = 32$).

to compare combined litter treatments. When necessary, data were log-transformed in order to conform to assumptions of normality and homogeneity of variance. A *P*-value of 0.05 or less was considered statistically significant. All tests were performed using SAS GLM procedures (SAS Institute Inc., 2003).

3. Results

Average earthworm biomass in the maize fields (2.26 g AFDW m^{-2}) and adjacent riparian buffer strips (2.19 g AFDW m^{-2}) did not differ significantly, although a substantial (albeit marginally non-significant) difference was found within two of the fields (Table 1). More specifically, earthworm populations and biomass were twice as high ($P = 0.07$) in the maize than in the switchgrass buffer strip in Field 2, and twice as low ($P = 0.08$) in the maize than in the hay buffer strip in Field 3. A total of four endogeic (*Aporrectodea turgida*, *A. tuberculata*, *A. rosea* and *Allolobophora chlorotica*), one anecic (*Lumbricus terrestris*) and one limicolous (*Eiseniella tetraedra*) earthworm species were found (Table 1). Both NO_3^- concentrations and net nitrification rates were significantly higher in the maize fields than in the buffer strip, whereas sampling location had no significant effect on other soil properties (Table 2).

Non-amended soils, and soils incubated in C_2H_2-enriched atmospheres, had the lowest N_2O production rates (<0.2 nmol N_2O g^{-1} h^{-1}) and showed no effect of sampling location (Fig. 1). When amended with $H_2O + C_2H_2$, N_2O production was six times higher ($P < 0.05$) in soils from buffer strips than from maize fieldss. A similar pattern was observed in soils amended with $NO_3^- + H_2O + C_2H_2$, with higher ($P < 0.01$) rates occurring in the buffer strips. N_2O production was highest when soils were amended with glucose + $H_2O + C_2H_2$, or with glucose + $NO_3^- + H_2O + C_2H_2$, but the effect of sampling location was not significant for these two trials.

Total C was higher in litters from woody species than in litters from agronomic crops (Table 3). Among agronomic crop litters, alfalfa was relatively nutrient-rich whereas switchgrass was relatively nutrient poor. Among woody litters, apple litter was relatively rich in total N and K, whereas *Rhododendron* litter was relatively poor in total N, P and K but relatively rich in total Na, Ca and Mg. Among agronomic crop litters, alfalfa was relatively rich in crude lipids and sugars, and relatively poor in crude cellulose and lignin fractions. In contrast, woody litters did not differ markedly among themselves in terms of proximate C fractions. Acidity was generally higher, and acid buffering capacity lower, in litters from woody species than from agronomic crops. Total phenolics, hydrolysable and condensed tannins were markedly higher in litters from woody species than from agronomic crops.

Repeated measures ANOVA revealed a significant main effect of litter amendment ($P < 0.001$), of time ($P < 0.001$) and of time x litter amendment interactions ($P < 0.001$) on earthworm biomass. At the conclusion of the five-week trial, earthworm biomass was higher in the alfalfa than in the switchgrass and corn litter treatments

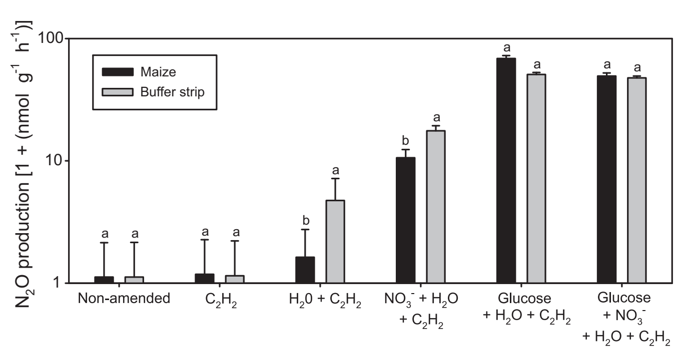

Fig. 1. Effect of sampling location (maize agroecosystem vs. riparian buffer strip) on soil nitrous oxide (N_2O) production (note the log scale) measured under six sets of experimental conditions. Different lowercase letters within the same cluster of bars denote significant differences between sampling locations ($P < 0.05$, $n = 4$); vertical lines = 1 S.D.

Table 2
Physico-chemical properties of soils sampled in four cultivated maize fields and adjacent uncultivated riparian buffer strips, near the Town of Bedford, Canada. Soil texture was sandy loam in fields #1 and #2, and clay in fields #3 and #4.

	Maize		Buffer strip
Total C (%)	3.13	N.S.	2.64
Total N (%)	0.26	N.S.	0.23
C/N ratio	11.4	N.S.	10.6
NH_4^+-N (mg kg^{-1})	2.45	N.S.	3.11
NO_3^--N (mg kg^{-1})	7.20	$P < 0.05$	4.28
Net ammonification (mg kg^{-1} mo^{-1})	−2.02	N.S.	−2.55
Net nitrification (mg kg^{-1} mo^{-1})	7.04	$P < 0.001$	1.13
pH	6.66	N.S.	6.66
Moisture (%)	26.3	N.S.	26.7

Table 3
Chemical attributes of six litter types used in the earthworm feeding trial, based on four categories of chemical assays: (i) elemental concentrations, (ii) proximate C fractions, (iii) acidity and acid buffering capacity, and (iv) phenolic substances.

	Agronomic species			Woody species		
	Alfalfa	Corn stover	Switchgrass	Rhododendra	Oak	Apple
(i)						
Carbon (mg g^{-1})	442.1	446.8	467.6	496.8	498.8	495.8
Nitrogen (mg g^{-1})	30.71	13.80	8.52	5.60	8.03	14.94
Carbon:nitrogen ratio	14.4	32.4	54.9	106.6	62.1	33.2
Potassium (mg g^{-1})	11.51	20.22	6.52	2.19	4.19	6.76
Phosphorus (mg g^{-1})	2.70	2.24	0.82	0.36	1.16	1.15
Sodium (mg g^{-1})	1.85	0.07	0.09	0.29	0.21	0.07
Calcium (mg g^{-1})	15.90	4.47	3.38	18.55	12.13	16.16
Magnesium (mg g^{-1})	4.10	1.65	0.94	2.48	1.17	1.87
(ii)						
Crude lipids (%)	16.7	3.3	4.1	20.4	17.1	18.1
Crude sugars (%)	41.7	20.7	20.3	33.6	31.7	32.1
Crude cellulose (%)	20.5	42.6	37.9	19.7	20.3	20.9
Crude lignin (%)	20.8	31.7	37.0	26.1	30.6	28.6
(iii)						
pH	5.84	6.81	6.08	5.01	4.65	5.44
Titratable acids (meq g^{-1})	0.081	0.010	0.017	0.087	0.096	0.043
Titratable bases (meq g^{-1})	1.00	1.06	1.11	0.80	0.54	0.76
Titratable acid/base ratio	0.081	0.009	0.015	0.108	0.177	0.057
Ash bases (meq g^{-1})	0.130	0.760	0.915	0.180	0.535	0.345
Acids/ash bases ratio	0.620	0.013	0.019	0.481	0.179	0.125
(iv)						
Total phenolics (mg g^{-1})	1.73	1.58	1.85	5.50	5.93	4.20
Hydrolysable tannins (mg g^{-1})	0.00	2.44	1.91	9.79	35.36	31.69
Condensed tannins (mg g^{-1})	0.00	0.00	0.00	19.60	4.80	2.02

(Fig. 2a), and higher in the oak than in the *Rhododendron* and apple litter treatments (Fig. 2b). Within the alfalfa mixtures, earthworm biomass was higher with oak and *Rhododendron* than with apple litter (Fig. 2c). Within the corn stover and switchgrass mixtures, earthworm biomass was higher with oak than with *Rhododendron* or apple litters (Fig. 2d and e). When only the agronomic species were considered, significant linear regressions were found between RGR and C:N ratio, and between RGR and lignin content of the litters (Table 4). When all plant species were considered, a significant multiple regression was found between RGR, lignin and hydroliz-

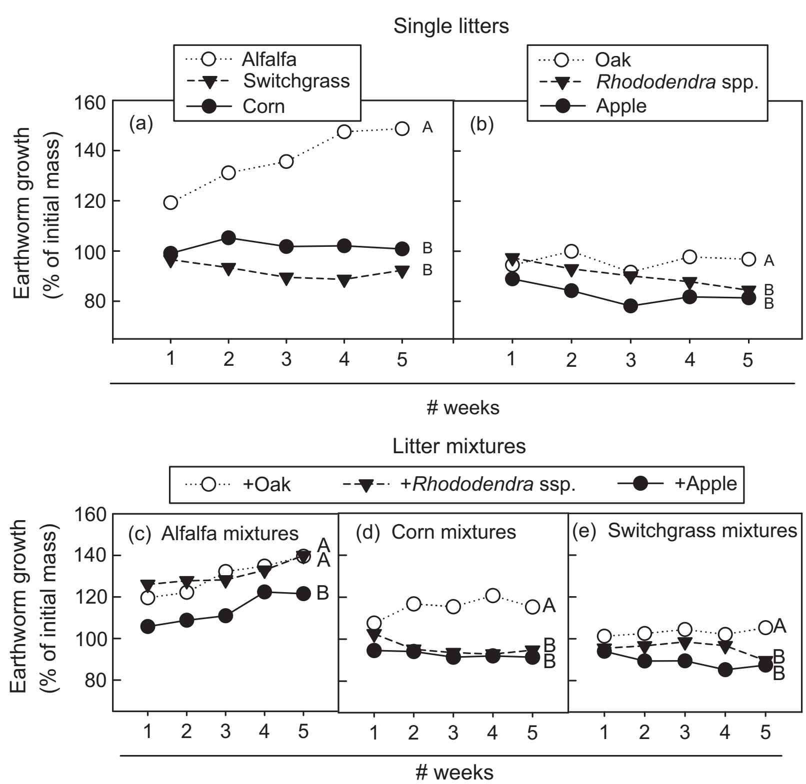

Fig. 2. Effects of 15 different litter amendments (1 g $week^{-1}$) on earthworm growth (depurated biomass) over a five week feeding trial. Treatments are divided as (a) agricultural crop litters, (b) woody plant litters and (c–e) alfala, corn and switchgrass litters respectively mixed with each woody plant litter. Values represent the mean of 10 replicates per treatment ($N = 150$). Different uppercase letters within each frame represent treatments in which final earthworm biomass ($t = 5$ weeks) differed significantly ($P < 0.05$).

Table 4
Significant simple and multiple regression equations relating leaf litter chemical attributes [C:N ratio, lignin (% dry wt) and hydrolysable tannins ($mg\,g^{-1}$)] to the mean relative growth rates (RGR) of earthworms over a five week feeding trial. RGR values are multiplied by 10^3 in order to eliminate multiple zero values from the regression coefficients.

Agronomic species	$RGR \times 10^3 = -3.22\,(C{:}N) + 137.61$	$R^2 = 0.68$	$P < 0.001$
Agronomic species	$RGR \times 10^3 = -8.83\,(lignin) + 293.04$	$R^2 = 0.82$	$P < 0.001$
All species	$RGR \times 10^3 = -9.29\,(lignin) - 46.69\,(tannin_{Hydr.}) + 1.51\,(lignin \times tannin_{hydr.}) + 295.23$	$R^2 = 0.68$	$P < 0.001$

able tannins, which included a significant interaction term between the two explanatory variables (Table 4).

N_2O production in earthworm–litter–soil mixtures amended with glucose + NO_3^- + H_2O + C_2H_2 (Fig. 3) was 2–4 times higher (80–170 nmol $N_2O\,g^{-1}\,h^{-1}$) than in plain soils amended in the same way (i.e., Fig. 1). Orthogonal contrasts revealed that the average N_2O production of litter treatments that included corn stover was lower ($P < 0.001$) than those that included alfalfa or switchgrass litters. Similarly, the average N_2O production of litter treatments that included oak litter was lower ($P = 0.03$) than those that included apple or *Rhododendron* litters.

4. Discussion

4.1. Earthworm abundances

Our data show inconsistent differences in earthworm abundances between maize fields and riparian buffer strips. It is notable that earthworm abundance was lower in a buffer strip planted with switchgrass, and higher in a buffer strip planted with hay. This observation is consistent with the chemical characteristics of switchgrass litter (discussed below) and with results from the earthworm–litter–soil mixtures, in which earthworm growth rates were lower with switchgrass than with corn stover or alfalfa. Taken collectively, these results offer presumptive evidence that earthworm abundance in buffer strips can be influenced by plant community composition.

4.2. Nitrification, N_2O production and potential denitrification

Given the high spatial variability in N_2O production and denitrification rates that are typically observed in the field (Hefting et al., 2006), all measurements in this study were performed on homogenized soil samples in a controlled laboratory environment, so as to allow comparisons based solely on chemical and microbial properties of the soil samples. While values cannot be extrapolated to field conditions, the data can be used to compare the relative magnitude of N_2O production from each sampling location, and to infer the relative magnitude of potential denitrification when soil moisture, NO_3^- and reduced C supply are not limiting.

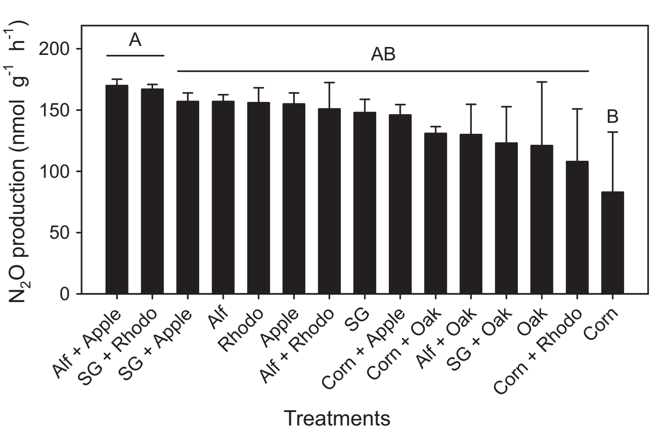

Fig. 3. Post-feeding trial nitrous oxide (N_2O) production. Each soil sample was amended with glucose, NO_3^- and H_2O, and incubated in a C_2H_2-enriched atmosphere. Different lowercase letters denote significant differences between litter treatments ($P < 0.05$, $n = 3$); vertical lines = 1 S.D. Alfalfa, *Rhododendra* spp. and switchgrass treatments are abbreviated as Alf, Rhodo and SG along the abscissa.

Lower net nitrification in the buffer strips may be due to low gross NO_3^- production rates, or to high gross NO_3^- consumption rates (Bradley, 2001). If the latter is true, then it is possible that substantial amounts of the consumed NO_3^- is subsequently reduced by denitrifying bacteria. Our data do not support this conjecture, however, given the lack of increase in N_2O production in the presence of C_2H_2. Rather, this result suggests that N_2O production in the unamended soil arose mainly from chemoautotrophic nitrification rather than heterotrophic denitrification (Firestone and Davidson, 1989).

N_2O production arising from chemoautotrophic nitrification is expected to dominate under aerobic conditions, whereas N_2O production arising from heterotrophic denitrification is expected in oxygen depleted soils (Firestone and Davidson, 1989). Accordingly, water-amendments resulted in a sixfold increase of N_2O production in the buffer strip soil samples (Fig. 1), but not in soil samples from the maize fields. Buffer strip soils are more likely to experience periodic water saturation as a result of their downslope position and of periodic swells in stream water levels. Our data suggest, therefore, that these periodic swells may have conditioned microbial communities in buffer strips to efficiently switch to their anaerobic respiratory pathway when soil moisture suddenly increases. This needs to be confirmed by monitoring denitrification in the field at a fine temporal scale that would establish whether buffer strips are more prone to "hot intervals" of high denitrification during wetting events.

Denitrification was limited by the availability of NO_3^- in both buffer strips and maize fields, as the addition of NO_3^- + H_2O + C_2H_2 resulted in a four-fold increase in N_2O production over that measured with the addition of H_2O + C_2H_2 (Fig. 1). The fact that denitrification with NO_3^- additions remained greater in buffer strip soils may reflect higher microbial biomass due to higher plant litter inputs and lower soil disturbance. Alternatively, this result may be because of stronger NO_3^- limitation in buffer strip soils due to lower nitrification rates (Table 2). The addition of glucose, with or without NO_3^-, resulted in the highest potential denitrification rates (Fig. 1), which suggests that the most limiting factor controlling denitrification in both sampling locations was the availability of labile organic C to drive heterotrophic metabolism.

Taken collectively, our data imply that N_2O emission rates in the field at the time of sampling were several orders of magnitude lower than potential denitrification rates, and that these potential rates may be higher in buffer strips than in maize fields, depending on the moisture or NO_3^- status of these soils. Results from our field survey of earthworm populations failed to confirm, however, that these differences in potential denification between sampling locations could be due to differences in earthworm densities.

4.3. Earthworm growth and potential denitrification in earthworm–litter–soil mixtures

Because feeding preferences vary among earthworm species (Neilson and Boag, 2003), our study focused exclusively on *L. ter-*

restris. This species is among the most abundant and widespread of exotic earthworms in Canada, and was present in each of the sampled fields. Although it was numerically less abundant than species such as *A. turgida*, it has the largest individual biomass of all species. It is one of only two anecic species in Canada, and is renowned for its ability to comminute large quantities of leaf litter and to burrow to depths of 2–3 m (Addison, 2009). Among the sampled earthworm species, *L. terrestris* is, therefore, likely to have the greatest impact on surface litter transformations and to affect the greatest soil volume. Accordingly, if riparian buffer strips were designed to reduce the growth of *L. terrestris* so as to ostensibly minimize NO_3^- leaching (Costello and Lamberti, 2008) or potential denitrification, our results suggest that woody tree or shrub species should be included whereas perennial legume species such as alfalfa should be avoided.

In keeping with its high concentrations of nutrients and simple sugars, and its low concentration of cellulose and lignin, alfalfa litter had a marked advantage in increasing earthworm growth over the other litter types. Alfalfa also had high crude lipid concentrations, as did litter from the woody species, but we suspect the former to be associated with metabolic (e.g., carotenoids) or storage (e.g., triglycerides) lipids, and the latter to be those associated with epicuticular waxes that make woody leaves less palatable to earthworms. Among the three agronomic crop litters, switchgrass was the least favourable for earthworm growth. Accordingly, this species had relatively high lignin, high total phenolics and low foliar nutrient concentrations, all of which are indicative of low nutritional quality.

Acidity, titratable acids and bases, titratable acid/base ratios, and ash bases have been used to distinguish "mull-forming" (high palatability) from "mor-forming" (low palatability) leaf litters (Howard and Howard, 1990). As expected, woody litters displayed greater acidifying characteristics than corn stover or switchgrass. The relatively high acidity of alfalfa litter may be due to the fact that this litter was still green, therefore rich in organic acids. It thus appears that the high nutritional value of alfalfa litter counteracted its acidifying nature in promoting the growth of earthworms.

Previous studies (e.g., Hendriksen, 1990) have shown that phenolic substances are negatively correlated with litter preference by detritivore earthworms. Condensed tannins, for example, form stable cross-links with litter protein making them extremely resistant to further degradation (Joanisse et al., 2009). We assayed more total phenolics, hydrolysable and condensed tannins in woody species than in the agronomic crop litters. Apple litter, which was the most detrimental to earthworm growth when applied either singly or in mixtures, showed the lowest concentrations of total phenolics and condensed tannins among woody litters. In fact, it is difficult to discern from our data what aspects of apple litter predisposed it to be of poor nutritional quality. For example, among the woody species the highest concentrations of N and P and the lowest acid/base ratios were found in apple litter, which is inconsistent with its negative effect on earthworm growth. Likewise, the variables found by multiple regression to be the best negative predictors or RGR (i.e., lignin and hydrolysable tannins) were higher in oak than in apple litter. We assume, therefore, that low RGR with apple litter was due to some other chemical characteristic, for example specific monoterpenes that may act as repellents (Vourc'h et al., 2002).

The fact that potential denitrification rates in earthworm–litter–soil mixtures were substantially higher than in plain soil samples amended in the same way, could either be due to litter amendments increasing soil available C, or to earthworm-mediated increases in denitrification. The latter would be consistent with other reports that suggested greater denitrification in earthworm-worked soil (Matthies et al., 1999; Borken et al., 2000; Speratti and Whalen, 2008). Regardless of the reason, our data underline the fact that different litter types interact differently with earthworms in significantly increasing potential denitrification. In this regard, the choice of plant species in designing riparian buffer strips remains difficult. For example, apple trees and switchgrass seem well suited to reduce earthworm biomass, which could potentially reduce NO_3^- leaching. On the other hand, these two species are not the best choice for mitigating denitrification. Furthermore, our study focused exclusively on litter quality as the main determinant of earthworm biomass and potential denitrification. It is likely, however, that plants exert an important control on these two variables through other mechanisms than litter quality. For example, slow growing (e.g., red oak) or leguminous (e.g., alfalfa) plant species are less demanding on soil N and may therefore stimulate potential denitrification more so than fast growing species (e.g., corn or switchgrass) under field conditions.

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