Soil Biology & Biochemistry 40 (2008) 1004–1010

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Short Communication

Earthworms, soil mineral nitrogen and forage production in grass-based hayfields

Nikita S. Eriksen-Hamel, Joann K. Whalen*

Department of Natural Resource Sciences, Macdonald Campus, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9

Received 13 July 2007; received in revised form 15 November 2007; accepted 17 November 2007
Available online 18 December 2007

Abstract

This study was designed to address how earthworm activity influences soil mineral nitrogen (N), plant N uptake and forage yield in grass-based hayfields. Earthworm populations were reduced by applying carbaryl pesticide to the experimental field plots every 2-weeks, effectively eliminating the earthworms for up to 12-weeks from May to August. Grass yields and tissue N concentrations were measured every 2 weeks, and the soil mineral N concentration determined at the final harvest. Reducing earthworm populations for up to 12-weeks did not affect grass yield or N uptake. However, regression analysis showed that plots with undisturbed earthworm populations had higher soil N by 0.8 kg N ha^{-1} per week, representing mineralization of about 10 kg N ha^{-1} during the 12-week study. This was a fraction of the fertilizer N recommendation (75 kg N ha^{-1}) for grass-based hayfields in this region. Therefore, the increase in soil mineral N from earthworm activity was small, relative to the N requirements of the hayfield.

Keywords: Carbaryl; Earthworm activity; Forage; Manipulation experiment; Nitrogen mineralization; Population reduction

Earthworms can contribute to the mineral N pool through interactions with soil microbial communities; as well, mineral N is excreted (urine and mucus) by active earthworms and released from dead earthworm tissues (Blair et al., 1995; Willems et al., 1996; Whalen et al., 1999; Hodge et al., 2000). The contribution of earthworms to the soil mineral N pool was estimated to represent 11–30% of the crop N requirements in cultivated maize agroecosystems (Whalen and Parmelee, 2000), suggesting that more earthworm activity could stimulate N uptake and primary production (Edwards and Bohlen, 1996; Scheu, 2003). Altering the size of earthworm populations can provide insight into how earthworms affect soil mineral N pools and plant growth (Bohlen et al., 1999; Subler et al., 1997).

Carbaryl is classified as a broad-spectrum insecticide of the carbamate group that causes acute poisoning by inhibiting acethylcholinesterase activity in earthworms and other invertebrates and vertebrates. Potter et al. (1990) found that applying carbaryl to turfgrass at a rate of 0.9 g a.i. m^{-2} reduced earthworm numbers and biomass by 90% for a period of 3-weeks. Non-target soil organisms (bacteria, fungi, protists, bacterivorous nematodes, fungivorous nematodes, mites) inhabiting the litter or surface soil layers (0–10 cm) were not affected by carbaryl applications of 0.3–1.2 g a.i. m^{-2} (Stegeman, 1964; Ingham et al., 1994; Childers et al., 2001). Carbaryl is degraded by bacteria that produce amidases, so it is a substrate that could stimulate bacterial activity (Elsayed et al., 1993; Kay-Shoemake et al., 2000), but there was no change in soil mineral N pools (NH_4-N and NO_3-N) following carbaryl application to maize agroecosystems (Ingham et al., 1994). Collembola populations were reduced for 4-weeks or longer when carbaryl was sprayed on soils, turfgrass or tree foliage at rates of 1.0–5.0 g a.i. m^{-2}, but recovered to pre-treatment levels after a few months (Stegeman, 1964; Dean et al., 1976; Potter et al., 1990; Liang et al., 2007). Since carbaryl is toxic to earthworms and has a negligible effect on most non-target soil organisms, we assumed that it could be used to manipulate earthworm populations and infer processes controlled by earthworms in the field.

Previously, we used carbaryl to reduce the naturally occurring earthworm populations in a cultivated agroecosystem, and then added earthworms into field enclosures at

*Corresponding author. Tel.: +1 514 398 7943; fax: +1 514 398 7990.
E-mail address: joann.whalen@mcgill.ca (J.K. Whalen).

0038-0717/$ - see front matter
doi:10.1016/j.soilbio.2007.11.013

known densities (Eriksen-Hamel and Whalen, 2007). Enclosures with larger earthworm populations had more mineral N, microbial biomass N and greater soybean grain yield, but results were not consistent in the second year due to poor survival of introduced earthworms. The present study used a different approach to examine the effect of earthworms on soil mineral N and plant growth in grass-based hayfields. Plots with naturally occurring earthworm populations were compared to plots where earthworm populations were reduced with carbaryl for 2–12-weeks during the growing season.

The study was conducted in mowed, unfertilized grass-dominated hayfields (less than 5% legumes) on the Macdonald Research Farm of the McGill University, Ste-Anne-de-Bellevue, Québec, Canada (45°25′N, 73°56′W). The hayfield studied in 2004 was dominated by smooth brome (*Bromus inermis* L.) and Kentucky bluegrass (*Poa pratensis* L.). In October 2004, the farm manager ploughed down this hayfield so that a maize crop could be planted in 2005. We selected a nearby hayfield on the same soil type, but with a different botanical composition—the dominant grasses were timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium prenne*) and smooth brome (*B. inermis* L.)—to repeat the experiment in 2005. Throughout this paper, the hayfields will be referred to by the dominant species present; *Bromus–Poa* in 2004, and *Phleum–Lolium* in 2005. The soil was a loam, classified as a mixed, frigid typic endoquent, with pH (H_2O) of 7.2 and containing between 56 and 133 g organic $C\,kg^{-1}$. Further details about the hayfields, soils and climate during the study period were reported by Eriksen-Hamel and Whalen (2006).

Experimental plots were arranged in a randomised complete block design with seven pesticide treatments replicated in four blocks ($7 \times 4 = 28$ experimental plots). Each plot measured $3 \times 3\,m^2$, with a 1 m buffer zone between plots and 5 m space between blocks. The pesticide treatments were intended to reduce earthworm populations in hayfields for 0, 2, 4, 6, 8, 10 and 12-weeks during the growing season. This was accomplished by applying carbaryl (Sevin®) at a rate of 5.0 g a.i. m^{-2}, starting on 18 May 2004 and 27 April 2005 in plots where earthworm populations were reduced for 12-weeks, and ending on 27 July 2004 and 6 July 2005 (plots with earthworm populations reduced for 2-weeks). Once the treatment was established, carbaryl was applied regularly (every 2-weeks) to reduce the number of immigrant and newly hatched earthworms in the plot. Thus, the 12-week treatment received 6 applications of carbaryl during the growing season, the 10-week treatment received 5 applications, and so on. Carbaryl (1.0 g a.i. m^{-2}) was also applied once a month to the plot perimeter (0.5 m strip) to eliminate earthworms migrating between plots.

The hayfields were mowed and forage was removed from the site before the first pesticide application. Beginning on 31 May 2004 and 11 May 2005, the plots were mowed 6 times, at 2-week intervals, to a height of 5 cm using a hand-pushed lawnmower with a collection bag. The final mowing was on 9 August 2004 and 18 July 2005. Forage from each plot was weighed (fresh weight) after mowing and a sub-sample (about 200 g) was used to determine the moisture content and calculate the forage production on a dry matter (DM, $g\,m^{-2}$) basis. Total forage yield ($g\,DM\,m^{-2}$) was the mass of forage collected from each plot during the growing season. Dried plant tissue was ground with a Wiley mill (<1 mm mesh), digested with H_2SO_4/H_2O_2 (Parkinson and Allen, 1975) and the tissue N concentration ($mg\,N\,kg^{-1}$) was analysed with a Lachat Quick Chem autoanalyzer (Lachat Instruments, Milwaukee, WI, USA). The N uptake ($g\,N\,m^{-2}$) was the sum of N removed from each plot in mowed forage (forage production × tissue N concentration, measured every 2-weeks) during the growing season.

To evaluate the seasonal fluctuations in earthworm population, four soil pits ($30 \times 30\,cm^3$ to a depth of 20 cm) were excavated every 2-weeks, within 2 days of mowing the

Table 1
Earthworm populations and biomass (fresh weight (FW) and ash-free dry weight (AFDW)) in each hayfield at final harvest as affected by carbaryl[a,b]

Treatment	Population (individuals m^{-2})	Biomass ($g\,FW\,m^{-2}$)	Biomass ($g\,AFDW\,m^{-2}$)
Bromus–Poa hayfield			
Background	54±34a	13.9±7.8a	1.5±0.8a
Plots without carbaryl	28±17a	9.5±3.9a	0.6±0.2a
Plots with carbaryl[b]	5.6±3.7b	0.3±0.2b	0.01±0.001b
% Reduction from carbaryl	80	97	98
Phleum–Lolium hayfield			
Background	180±49a	23.8±8.1a	1.6±0.5a
Plots without carbaryl	260±54a	21.6±1.8a	1.7±0.3a
Plots with carbaryl[b]	40±20b	2.9±1.3b	0.1±0.2b
% Reduction from carbaryl	85	86	92

[a]Values are the mean±S.E.; $n = 8$ for background populations (earthworms collected from the hayfield surrounding the experimental site), $n = 4$ for experimental plots; values followed by different letters were statistically different ($P<0.05$, *t*-test).

[b]Plots received single or multiple carbaryl applications to reduce earthworm populations for 2, 4, 10 and 12 weeks during the study. All plots had received a carbaryl application (5 g a.i. m^{-2}) 2 weeks before earthworms were collected.

hayfield. Earthworms were collected from this soil by hand sorting. Dilute formalin solution (0.5% formaldehyde) was poured into the bottom of the pit until saturated and deeper-dwelling earthworms were collected about 15 min after the excess formalin solution had drained through macropores. These soil pits were located between replicated blocks, at least 1.5 m away from experimental plots to avoid the perimeter where carbaryl was applied. Earthworm numbers, species and the biomass, expressed as fresh weight (FW) and ash-free dry weight (AFDW), were recorded in the laboratory After the final mowing, earthworms were collected from soil pits in the middle of four plots that never received carbaryl. We also selectively sampled two plots that had received carbaryl applications to reduce earthworm populations for most or all of the growing season (10- and 12-week treatments) and two plots where carbaryl was applied once or twice during the growing season (2- and 4-week treatments). It should be noted, however, that all four plots received a carbaryl application 2-weeks before earthworms were collected. The efficiency of carbaryl at reducing earthworm populations was calculated as: [(earthworms in control plots earthworms in carbaryl treated plots)/earthworms in control plots]. We also collected earthworms from soil pits located about 10 m to the west ($n = 4$) and east ($n = 4$) of the experimental plots, which were designated as the background earthworm population. A t-test was used to compare the earthworm populations in the background area, control plots and carbaryl treated plots. A representative soil sample (four cores, 2 cm diameter to a depth of 15 cm) was taken from each plot after the final mowing. Soil mineral N (NO_3-N + NH_4-N) was determined in 2 M

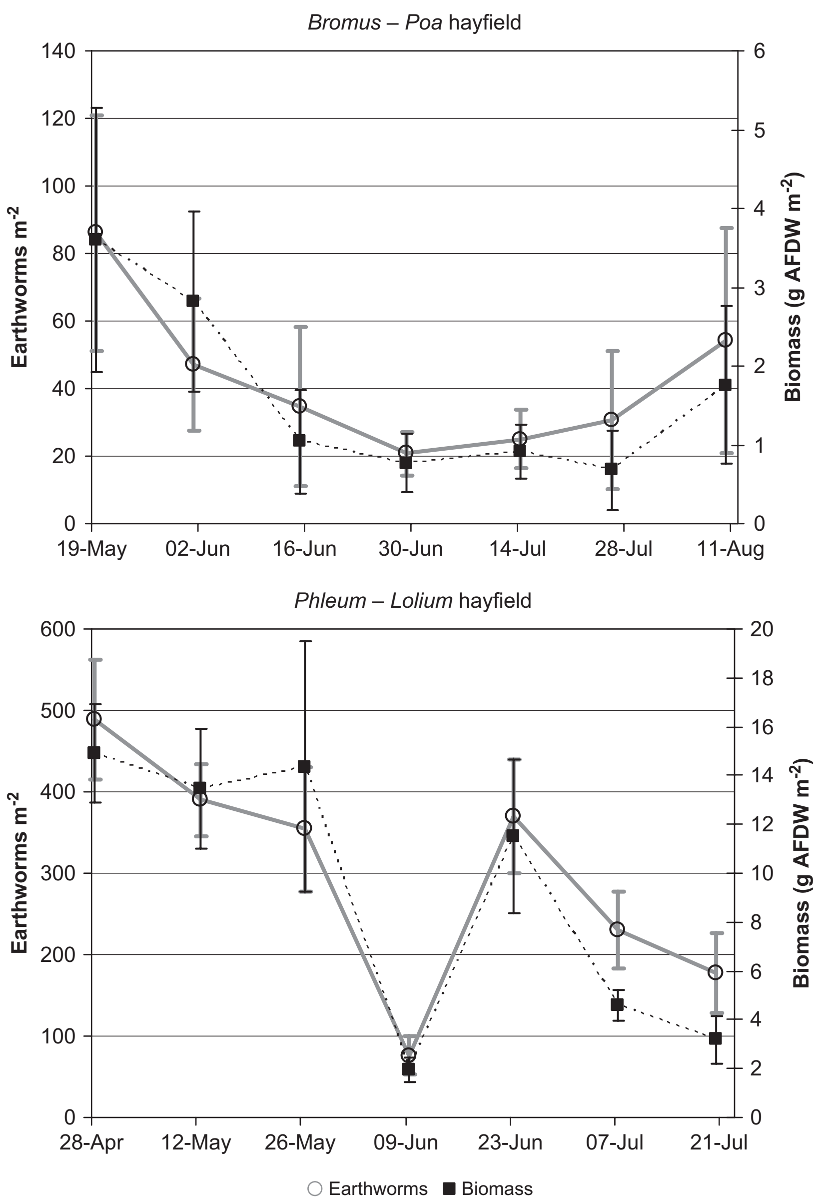

Fig. 1. Naturally occurring earthworm populations (○) and ash-free dry weight (AFDW) biomass (■) in unfertilized *Bromus–Poa* and *Phleum–Lolium* hayfields. Values shown as mean ± S.E. Scales were different due to a larger population of naturally occurring earthworms in the *Phleum–Lolium* hayfield.

KCl extracts (1:10 soil:extractant) and analysed on a Lachat Quick Chem auto-analyzer (Maynard and Kalra, 1993).

The effect of carbaryl treatments on total forage yield, N uptake and soil mineral N was evaluated using one way ANOVA analysis and linear regressions were fitted using SAS software (SAS System 9.1, SAS Institute Inc., Cary, NC).

The earthworm population in the *Bromus–Poa* hayfield was dominated by *Aporrectodea caliginosa*, with a few *Lumbricus terrestris* and *Octolasion tyrtaeum*. In the *Phleum–Lolium* hayfield, the dominant species were *A. caliginosa* and *Aporrectodea rosea*. Carbaryl applications reduced the number of earthworms present, and biomass was 86–98% lower in the plots that received carbaryl than those that were not treated (Table 1). At the end of each growing season, the number and biomass of earthworms in experimental plots without carbaryl was similar to the background earthworm population collected from the hayfield surrounding the experimental site (Table 1). We concluded that carbaryl applications were effective at reducing earthworm populations in these grass-based hayfields, and that the toxic effects of this insecticide were confined to experimental plots, making it a suitable biocide for manipulation studies. We assumed that carbaryl had a negligible effect on non-target soil biota and the processes that they regulate (i.e., N mineralization), but this remains to be verified in future experiments.

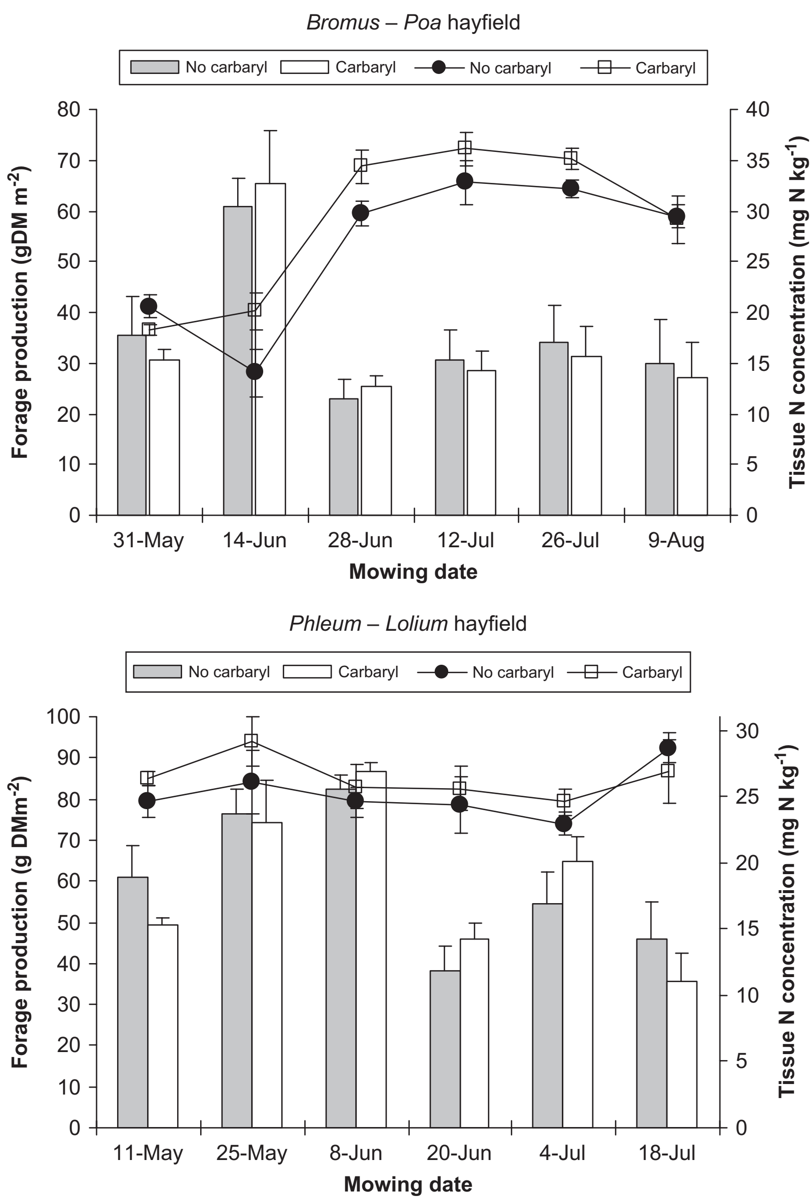

Fig. 2. Seasonal fluctuations in forage production (g DM m^{-2}, grey and white bars) and tissue N concentration (mg N kg^{-1}, ● and □ symbols plus lines) in unfertilized *Bromus–Poa* and *Phleum–Lolium* hayfields with naturally occurring earthworm populations (no carbaryl) and reduced earthworm populations (carbaryl, 12-week treatment). The carbaryl-treated plots received 6 applications of pesticide, starting 2-weeks before the first forage sample was collected and repeated every 2-weeks during the growing season. Values are the mean ± S.E. ($n = 4$).

More earthworms were collected in spring than in midsummer (Fig. 1), which is typical for this region (Whalen, 2004). The decline in earthworm populations was gradual from 19 May to 30 June 2004, with an increase in earthworm numbers from mid-July to mid-August (Fig. 1). Although earthworm populations were relatively stable during April and May 2005, there was a dramatic reduction in earthworm numbers and biomass between 26 May and 9 June 2005, which coincided with a period when the daily air temperature often exceeded 30 °C and there was no rain (Fig. 1). The disappearance of earthworms during hot, dry periods may indicate earthworm mortality or the retreat of earthworms to deep soil layers that were not sampled efficiently by a combination of hand-sorting and formalin extraction.

The seasonal fluctuations in earthworm populations could contribute to N cycling and plant growth as follows: (1) a decline in earthworm populations due to mortality could release N (from dead earthworm tissue) that is readily available for plant uptake (Whalen et al., 1999; Hodge et al., 2000), and (2) earthworm populations are stable or increase in size when soil conditions are favourable for their activity (foraging, burrowing, reproduction, etc.). Active earthworms excrete plant-available N compounds (in urine and mucus) and interact with microorganisms and other foodweb organisms involved in decomposition, N mineralization and nitrification processes (Edwards and Bohlen, 1996). However, fluctuations in naturally occurring earthworm populations (Fig. 1) were not correlated with forage production and tissue N concentration measured at 2-week intervals (Fig. 2). There was also no difference in forage production and tissue N concentrations among the experimental treatments, as illustrated for plots with unmodified earthworm populations (no carbaryl) and for plots where carbaryl was added every 2-weeks to reduce earthworm populations (Fig. 2). Thus, the total forage yield and N uptake in these grass-based hayfields was not affected by the carbaryl applications that reduced earthworm populations (Fig. 3).

A short-term reduction in earthworm activity or population size may have very little effect on plant growth, unless the soil is very nutrient poor and the earthworm-mobilized nutrient pool represents a sizeable fraction of the plant requirements (Lavelle et al., 2001). Due to the seasonal fluctuations in earthworm populations, their contribution to nutrient mineralization is probably greater in spring and fall than in the summer. An established hayfield is expected to have a well-developed rooting system that can extract water and nutrients from the soil profile, so the short-term disappearance of earthworms would probably have little impact on plant nutrition. However, removing earthworms for longer periods of time could affect processes like thatch removal, organic matter incorporation, aggregate and macropore formation in hayfields. Lavelle et al. (2001) noted that primary production and other ecosystem functions are buffered by the structural integrity of biogenic structures like casts and burrows, which can persist for some time after earthworms

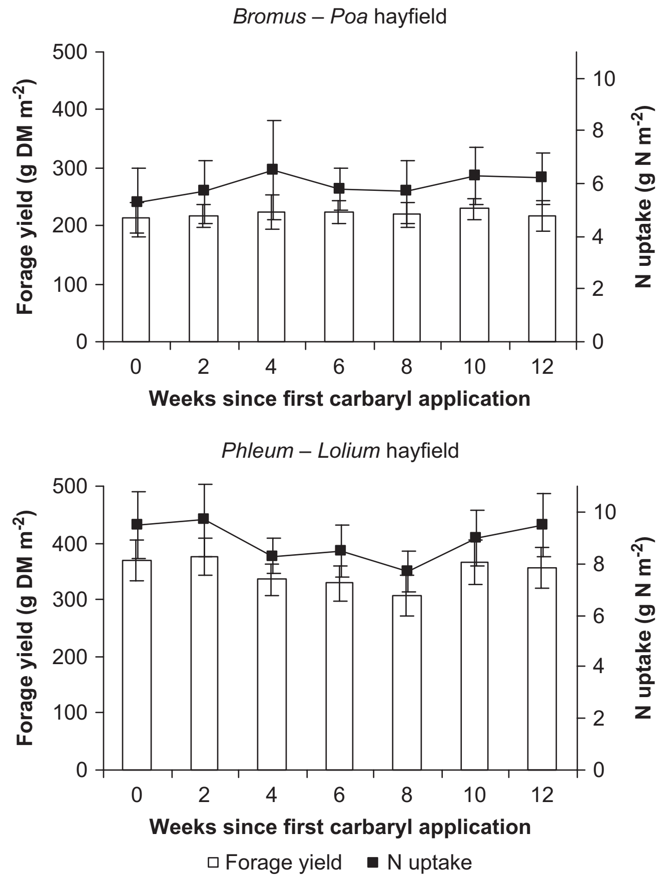

Fig. 3. Total forage yield (g DM m^{-2}, white bars) and N uptake (g N m^{-2}, ■ plus lines) in unfertilized *Bromus–Poa* and *Phleum–Lolium* hayfields, related to the number of weeks since the first carbaryl application to reduce earthworm populations. Values shown as mean ± S.E. ($n = 4$).

die. We have no data on the persistence of earthworm structures in Eastern Canada, although we expect that they would be gradually degraded by wetting/drying and freeze/thaw cycles. Further work is needed to investigate how biogenic structures created by earthworms may contribute to the ecosystem functioning in this region.

We found less soil mineral N in plots where earthworm populations were reduced by repeated carbaryl applications during a 12-week period, compared to untreated plots (Fig. 4). The regression equations describing these relationships indicate a decrease of 0.32–0.33 mg NO_3-N kg^{-1} and 0.32–0.38 mg mineral N kg^{-1} per $week^{-1}$ that earthworm populations were reduced. This is about 0.7–0.9 kg mineral N ha^{-1} per $week^{-1}$, based on a hectare furrow slice, and represents an extra 8–11 kg mineral N ha^{-1} during a 12-week period. This is only a fraction of the fertilizer N recommendation of 75 kg N ha^{-1} for hayfields in Quebec (Centre de Reference en Agriculture et Agroalimentaire du Québec (CRAAQ), 2003). Previously, we found that these grass-based hayfields respond to fertilizer N applications, as forage yield increased by 60–280 g DM m^{-2} when inorganic NPK fertilizer or dairy slurry was applied at a rate of 75 kg N ha^{-1} (Eriksen-Hamel and Whalen, 2006). We conclude that the increase in soil mineral N from

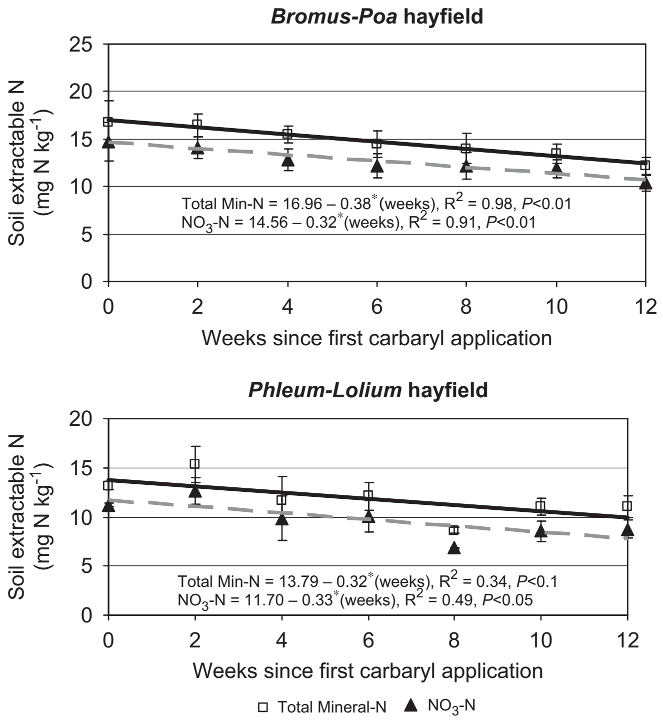

Fig. 4. Soil NO_3-N (▲) and mineral-N concentrations (□, both in mg N kg^{-1}) in unfertilized *Bromus–Poa* and *Phleum–Lolium* hayfields, related to the number of weeks since the first carbaryl application to reduce earthworm populations. Values shown are the mean ± S.E. ($n = 4$) and equations of the linear regression through mean values ($n = 7$).

earthworm activity was small, relative to the N requirements of the hayfield. Similar results were found in a Swiss grassland, where earthworm activity was artificially increased by manipulating population size and increasing soil moisture, however, there was no change in above-ground biomass production due to more earthworm activity (Zaller and Arnone, 1999). The contribution of earthworms to N mineralization might enhance N uptake by grasses during periods when earthworms are highly active or in sites with large earthworm populations, but this remains to be confirmed for grass-based hayfields in North America.

We would like to thank Jonathan Perreault, Arlette Lteif, You Jiao, Aleš Kulhánek, Simon Lacombe and Valérie Campeau for assistance with fieldwork. Helpful comments from two anonymous reviewers and the Chief Editor, Dr. John Waid, were greatly appreciated. Financial assistance was from the Natural Sciences and Engineering Research Council (NSERC), and the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT).

References

Blair, J.M., Parmelee, R.W., Lavelle, P., 1995. Influence of earthworms on biogeochemistry. In: Hendrix, P.F. (Ed.), Earthworm Ecology and Biogeography. CRC Press, Boca Raton, FL, USA, pp. 127–158.

Bohlen, P.J., Parmelee, R.W., Allen, M.F., Ketterings, Q.M., 1999. Differential effects of earthworms on nitrogen cycling from various nitrogen-15-labeled substrates. Soil Science Society of America Journal 63, 882–890.

Centre de Reference en Agriculture et Agroalimentaire du Québec (CRAAQ), 2003. Guide de Référence en Fertilisation, first ed. Quebec, Canada.

Childers, C.C., Villanueva, R., Aguilar, H., Chewning, R., Michaud, J.P., 2001. Comparative residual toxicities of pesticides to the predator *Agistemus industani* (Acari: Stigmaeidae) on citrus in Florida. Experimental and Applied Acarology 25, 461–474.

Dean, H.J., Colquhoun, J.R., Frisa, C.N., 1976. Some effects of carbaryl on mites, collembola and *Calosoma* sp. in an oak type forest. Environmental Entomology 5, 793–797.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms, third ed. Chapman & Hall, London, UK.

Elsayed, M.A., Elshanshoury, A.R., Swelim, M.A., Elsalam, S.S.A., 1993. Microbiological and physiochemical properties of carbamate insectides treated soil. Journal of Agronomy and Crop Science 170, 217–223.

Eriksen-Hamel, N.S., Whalen, J.K., 2006. Fertilization and mowing effects on unimproved mixed-species hayfields in Quebec, Canada. Crop Science 46, 1955–1962.

Eriksen-Hamel, N.S., Whalen, J.K., 2007. Impacts of earthworms on soil nutrients and plant growth in soybean and maize agroecosystems. Agriculture, Ecosystems and Environment 120, 442–448.

Hodge, A., Stewart, J., Robinson, D., Griffiths, B.S., Fitter, A.H., 2000. Plant N capture and microfaunal dynamics from decomposing grass and earthworm residues in soil. Soil Biology & Biochemistry 32, 1763–1772.

Ingham, E.R., Coleman, D.C., Crossley, D.A., 1994. Use of sulfamethoxazole–penicillin, oxytetracycline, carbofuran, carbaryl, naphthalene and temik to remove key organism groups in soil in a corn agroecosystem. Journal of Sustainable Agriculture 4, 7–30.

Kay-Shoemake, J.L., Watwood, M.E., Sojka, R.E., Lentz, R.D., 2000. Soil amidase activity in polyacrylamide-treated soils and potential activity toward common amide-containing pesticides. Biology anf Fertility of Soils 31, 183–186.

Lavelle, P., Barros, E., Blanchart, E., Brown, G., Desjardins, T., Mariani, L., Rossi, J.-P., 2001. SOM management in the tropics: why feeding the soil macrofauna? Nutrient Cycling in Agroecosystems 61, 53–61.

Liang, W., Beattie, G.A.C., Meats, A., Spooner-Hart, R., 2007. Impact on soil-dwelling arthropods in citrus orchards of spraying horticultural mineral oil, carbaryl or methidathion. Australian Journal of Entomology 46, 79–85.

Maynard, D.G., Kalra, Y.P., 1993. Nitrate and exchangeable ammonium nitrogen. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis Canadian Society of Soil Science. Lewis Publishing, Boca Raton, FL, USA, pp. 25–32.

Parkinson, J.A., Allen, S.E., 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Communication in Soil Science snf Plant Analysis 6, 1–11.

Potter, D.A., Buxton, M.C., Redmond, C.T., Patterson, C.G., Powell, A.J., 1990. Toxicity of pesticides to earthworms (Oligochaeta: Lumbricidae) and effect on thatch degradation in Kentucky Bluegrass turf. Journal of Economic Entomology 83, 2362–2369.

Scheu, S., 2003. Effects of earthworms on plant growth: patterns and perspectives. Pedobiologia 47, 846–856.

Stegeman, L.C., 1964. Effects of carbamate insecticide carbaryl upon forest soil mites and collembola. Journal of Economic Entomology 57, 803–808.

Subler, S., Baranski, C.M., Edwards, C.A., 1997. Earthworms additions increased short-term nitrogen availability and leaching in two grain-crop agroecosystems. Soil Biology & Biochemistry 29, 413–421.

Whalen, J.K., 2004. Spatial and temporal distribution of earthworm patches in corn field, hayfield and forest systems of southwestern Quebec, Canada. Applied Soil Ecology 27, 143–151.

Whalen, J.K., Parmelee, R.W., 2000. Earthworm secondary production and N flux in agroecosystems: a comparison of two approaches. Oecologia 124, 561–573.

Whalen, J.K., Parmelee, R.W., McCartney, D.A., VanArsdale, J.L., 1999. Movement of N from decomposing earthworm tissue to soil microbial and plant N pools. Soil Biology & Biochemistry 31, 487–492.

Willems, J.J.G.M., Marinissen, J.C.Y., Blair, J., 1996. Effects of earthworms on nitrogen mineralization. Biology and Fertility of Soils 23, 57–63.

Zaller, J.G., Arnone, J.A., 1999. Earthworm and soil moisture effects on the productivity and structure of grassland communities. Soil Biology & Biochemistry 31, 517–523.