

Applied Soil Ecology 9 (1998) 289-294

Applied Soil Ecology

Cognettia sphagnetorum (Enchytraeidae) and nutrient cycling in organic soils: a microcosm experiment

M.J.I. Briones^{a,*}, J. Carreira^b, P. Ineson^c

^a Departamento de Ecologia y Biologia Animal, Facultad de Ciencias, Universidad de Vigo, 36200 Vigo, Spain ^b Departamento de Biología Animal, Vegetal y Ecología, Facultad de Ciencias Experimentales, Universidad de Jaén, 23071 Jaén, Spain ^c Merlewood Research Station, Institute of Terrestrial Ecology, Grange-over-Sands, Cumbria LA11 6JU, UK

Received 30 August 1996; accepted 22 November 1996

Abstract

A microcosm experiment was carried out to investigate the role of enchytraeids in nitrogen and phosphorus mineralisation in an organic grassland soil. Soil cores were taken from a cambic stagnohumic gley with associated vegetation in the Moor House National Nature Reserve (UK), defaunated, separated into 3 cm layers and inoculated with *Cognettia sphagnetorum* (Vejdovsky) in microcosms, maintained at 15° C in the laboratory. The leaching of dissolved organic nitrogen (DON), ammonium and phosphorus (total, organic and soluble reactive phosphorus) was monitored over 8 weeks, and found to be significantly greater in the presence of *C. sphagnetorum*; this effect varied with soil depth, highest increases being found in the 0–3 and 3–6 cm soil layers. The pH in the leachates was also increased in the presence of *Cognettia*. The impacts of seasonal vertical distribution of enchytraeids on nutrient cycling are discussed, together with the implications of climate change in these systems. © 1998 Elsevier Science B.V.

Keywords: Enchytraeids; Cognettia sphagnetorum; Climate change; Microcosm

1. Introduction

Enchytraeids are frequently the major soil faunal component in moorland soils and their biomass can exceed that of any other faunal component in moorlands and montane grasslands (Coulson and Whittaker, 1978), either above- or below-ground, but biomass and energy flow data do little to inform us of their role in facilitating nutrient cycling in such ecosystems. Enchytraeids have been reported to influence both respiration and nutrient leaching in coniferous forest soils (Williams and Griffiths, 1989; Setälä et al., 1991; Haimi and Boucelham, 1991) with important positive effects on the mineralisation of inorganic N and P. There is also a growing realisation of the importance of the cycling of organic forms of N and P in nutrient poor soils, with mycorrhizae being associated with plant uptake of organic forms of major nutrients (Michelsen et al., 1996). The consequences of enchytraeid activity for the release of both organic and inorganic forms of these elements may be of considerable importance in these situations, and needs to be quantified.

^{*}Corresponding author. Tel.: 34 86 812584; fax: 34 86 812556; e-mail: mbriones@uvigo.es

^{0929-1393/98/\$19.00 © 1998} Elsevier Science B.V. All rights reserved. *P11* \$0929-1393(97)00055-3

Enchytraeids are frequently concentrated in the upper horizons (Springett et al., 1970) where organic matter accumulates and the majority of the absorptive roots occur. Changes in the temperature and water regimes, as forecast under future climate scenarios, have been shown to influence the population sizes and vertical distribution of enchytraeids in upland soils (Briones et al., 1997), and the consequences for nutrient turnover need to be assessed.

The aim of the current project was to investigate the effects of enchytraeids on nutrient mobilisation in these soils, in relation to soil depth, and to consider the implications of potential climate change. The organism chosen for study was *Cognettia sphagnetorum* (Vejdovsky) 1877, because this species dominates the faunal population at the study site, and more than 80% of the population is found within the top 3 cm of the soil (Coulson and Whittaker, 1978).

2. Materials and methods

2.1. Preparation and watering of microcosms

Eight intact soil cores with associated vegetation (Juncus squarrosus L. with Festuca ovina L., Deschampsia flexuosa (L.)Trin. and Polytrichum commune L.) were taken from a cambic stagnohumic gley near to the summit of Great Dun Fell (within the Moor House National Nature Reserve; NGR 710322), using 5 cm diam.×10 cm deep acrylic cylinders. Each core was placed in liquid nitrogen for 1 h, thawed and sliced into three layers, each of 3 cm, to a total depth of 9 cm. Individual layers were introduced into a separate microcosm inner container (Anderson and Ineson, 1982), resulting in a total of 24 experimental units. Half of the microcosms were inoculated with animals (see below) and designated as +animals treatments (A) with the remaining twelve referred as control treatments (C). The microcosms were incubated at 15°C for 8 weeks using a randomised block design.

Cognettia sphagnetorum organisms were extracted from cores taken from the same site using a modified wet funnel method (O'Connor, 1955) and introduced, 350 individuals per microcosm, into four replicates of each of the soil layers. This produced a population density similar to that seen under optimal conditions in the field (Briones et al., 1997).

Leaching was performed by gentle immersion in distilled water (200 cm³), draining under gravity, and re-application of the leachate to the surface of the soil twice to ensure thorough equilibration of mineralised nutrients between the soil and the leachate (Anderson and Ineson, 1982). In following samplings, the soil was leached with 100 cm³ of distilled water and allowed to soak for 1 h before draining. This procedure was repeated every two weeks over the experimental period of 8 weeks.

2.2. Chemical analyses of leachates

Leachates were analysed for pH by using a Pye Unicam pH meter and combination electrode. Samples were then stored at 2°C and analysed for phosphorus (soluble reactive P, total P and $PO_4^{3-}-P$) and NH₄⁺-N (for the last four weeks). For fractionation of dissolved phosphorus, we followed Ron Vaz et al. (1993) and Negrín et al. (1995). Dissolved inorganic phosphorus (DIP) was determined colorimetrically by the method of John (1970) after acidifying with 1 M HCl to precipitate extracted organic matter. When concentrations were less than 0.1 mg P l^{-1} we used the more sensitive malachite green method (Fernández et al., 1985). Another aliquot of leachate was subjected to a H₂O₂-H₂SO₄ wet digestion (Parkinson and Allen, 1975) and dissolved total P (DTP) was determined by the malachite green method. Dissolved organic P (DOP) was defined as DOP=DTP-DIP.

Mineral NH_4^+ –N was analysed using a Technicon continuous flow autoanalyser following the salicylate– hypochlorite method (Gentry and Willis, 1988). Dissolved organic nitrogen (DON) was estimated as the difference between NH_4^+ –N in digested aliquots and mineral NH_4^+ –N contents in the original leachates; NO_3^- concentrations were consistently insignificant. Phosphatase activity (incubation at pH 5.5) was assayed using *p*-nitrophenyl phosphate (PNP*p*) as substrate (Tabatabai and Bremer, 1969).

2.3. Statistical analysis

Leachate concentrations were converted to mgs leached and mean values and standard errors calculated. pH values were not transformed prior to analysis. Comparisons of mean leachate concentrations across the treatments and for the whole experimental period were made using analyses of variance (ANOVA).

3. Results

3.1. Nitrogen

N release from all microcosms varied with depth, being generally greater in the 0–3 cm layer. However,

leaching of NH_4^+ from the 3–6 cm layer in the A treatments increased during the course of the experiment, reaching values close to those of the 0–3 cm treatment by the 8th week (Fig. 1a). The soil fauna significantly increased NH_4^+ –N leaching (p<0.01), with greater release being observed in all three layers when compared with the controls.

Leachates from refaunated microcosms showed greater concentrations of DON than the controls (p<0.01) (Fig. 1b), with the greatest values being observed in the 3–6 cm layer at the end of the experimental period.



Fig. 1. Nitrogen release as ammonium (mg NH_4^+ 2 weeks⁻¹) (a) and dissolved organic nitrogen (mg DON 2 weeks⁻¹) (b) from microcosms containing soil from the 0–3 cm (\Diamond), 3–6 cm (\square) and 6–9 cm (\bigcirc) layers. Animal treatments are shown as solid symbols and controls as open symbols. Values are means with standard errors, n=4.



Fig. 2. Phosphorus release as dissolved total (a), dissolved inorganic (b) and dissolved organic phosphorus (c) from microcosms containing soil from the 0–3 cm (\Diamond), 3–6 cm (\Box) and 6–9 cm (\bigcirc) layers. Animal treatments are shown as solid symbols and controls as open symbols. Values are means with standard errors, n=4.

3.2. Phosphorus

Total and soluble reactive phosphorus (SRP) releases increased in the upper soil layers (0–3 and 3–6 cm) in the presence of *C. sphagnetorum*, with values becoming significantly greater after four weeks (p<0.01) (Fig. 2a,b). The lowest release rates for the entire experimental period were observed in the 6–9 cm depths in the defaunated systems.

No significant differences were observed in the organic phosphorus release at the beginning of the experimental period, but after 4 weeks, leachates from the refaunated microcosms had the greatest concentrations of DOP, which were consistent and significantly different (p<0.01) from the controls at the end of the experimental period (Fig. 2c).

No significant differences in soil acid phosphatase activity between control $(2.6 \,\mu\text{Mol} \text{ PNP/g h})$ and refaunated microcosms $(2.0 \,\mu\text{Mol} \text{ PNP/g h})$ were detected.

3.3. pH

Leachate pH in the absence of *Cognettia* gradually decreased during the course of the experiment, with the lowest values being observed at week 6 for the 6–9 cm layer (Fig. 3). However, in the presence of enchytraeids a significant reduction of leachate acidity (p<0.001) was observed after four weeks.

4. Discussion

The enchytraeids had a clear positive influence on nutrient release which varied with soil depth. The most dramatic increases in N and P release occurred in the 0–3 and 3–6 cm layers. Leachate concentrations of both inorganic N and P were increased in the presence of animals, which agrees well with the results of others (for example, Anderson et al., 1983; Huhta et al., 1988; Setälä et al., 1990 and Förster et al., 1995). However, the current work clearly demonstrated the importance of *C. sphagnetorum* in increasing soluble organic forms of N and P. Both DON and DOP leaching was significantly enhanced, indicating that organic matter turnover was accelerated by these organisms.

The mechanisms by which enchytraeids may affect nutrient cycling may be attributable to direct (e.g. excretory products, such as NH_4^+) or indirect effects (e.g. improving the soil structure for microbial mineralisation) (Anderson and Ineson, 1984). As saprovores, they ingest either pure organic substances (litter and plant debris) or a mixture of organic and mineral material. Because of their low assimilation efficiency they have to ingest large amounts of material and, as a result of this, large amounts of undigested material characterized by specific microbial communities, high microbial activity and high organic and nutrient availability is produced (Martin and Marinis-



Fig. 3. pH values from microcosms containing soil from the 0–3 cm (\Diamond), 3–6 cm (\square) and 6–9 cm (\bigcirc) layers. Values are means with standard errors, n=4.

sen, 1993). On the other hand, indirect effects through grazing activity may lead to the continuous renewal of active microbial populations (Sparling et al., 1981).

The impacts of climate change on the soil biota have received less attention than other components of ecosystems, and there have been few studies on the responses of soil fauna to climate change. However, our knowledge of the responses of certain soil faunal components to changes in temperature and moisture conditions suggest that these organisms will respond markedly to new climatic conditions. For example, the seasonal vertical migrations of C. sphagnetorum at the Moor House study site are clearly linked to air temperatures (Springett et al., 1970; Briones et al., 1997), with the animals going deeper into the soil during dry periods. The work presented here has shown that Cognettia increases the release of organic and inorganic N and P, and that climatically-induced vertical migrations of this species could have a marked influence on organic matter decomposition and nutrient provision in these soils.

References

- Anderson, J.M., Ineson, P., 1982. A soil microcosm system and its application to measurement of respiration and nutrient leaching. Soil Biol. Biochem. 14, 415–416.
- Anderson, J.M., Ineson, P., 1984. Interaction between microorganisms and soil invertebrates in nutrient flux pathways of forest ecosystems. In: Anderson, J.M., Rayner, A.D.M., Walton, D.W.H. (Eds.), Invertebrate-Microbial Interactions. Cambridge University Press, Cambridge, pp. 59–88.
- Anderson, R.V., Gould, W.D., Woods, L.E., Cambardella, C., Ingham, R.E., Coleman, D.C., 1983. Organic and inorganic nitrogenous losses by microbivorous nematodes in soil. Oikos 40, 75–80.
- Briones, M.J.J., Ineson, P., Piearce, T.G., 1997. Effects of climate change on soil fauna; responses of enchytraeids, Diptera Larvae and tardigrades in a transplant experiment. App. Soil Ecol. 6, 117–134.
- Coulson, J.C., Whittaker, J.B., 1978. Ecology of moorland animals. In: Heal, O.W., Perkins, D.F. (Eds.), Production Ecology of British Moors and Montane Grasslands. Springer, Berlin, pp. 52–93.
- Fernández, J.A., Niell, F.X., Lucena, J., 1985. A rapid and sensitive automated determination of phosphate in natural waters. Limnology and Oceanography 30, 227–230.
- Förster, B., Römbke, J., Knacker, T., Morgan, E., 1995. Microcosm study of the interactions between microorganisms and enchy-

traeid worms in grassland soil and litter. Eur. J. Soil Biol. 31(1), 21–27.

- Gentry, C.E., Willis, R.B., 1988. Improved method for automated determination of ammonia in soil extracts. Communications in Soil Science and Plant Analysis 19, 721–737.
- Haimi, J., Boucelham, M., 1991. Influence of a feeding earthworm *Lumbricus rubellus* on soil processes in a simulated coniferous forest floor. Pedobiologia 35, 246–256.
- Huhta, V., Setälä, H., Haimi, J., 1988. Leaching of N and C from birch leaf litter and raw humus with special emphasis on the influence of soil fauna. Soil Biol. Biochem. 20(6), 875–878.
- John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. Soil Science 109, 214– 220.
- Martin, A., Marinissen, J.C.Y., 1993. Biological and physicochemical processes in excrements of soil animals. Geoderma 56, 331–347.
- Michelsen, A., Schmidt, I.K., Jonasson, S., Quarmby, C., Sleep, D., 1996. Leaf 15N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. Oecologia 105, 53–63.
- Negrín, M.A., González, S., Hernández, J.M., 1995. P fractionation in sodium bicarbonate extracts of andic soils. Soil Biol. Biochem. 27, 761–766.
- O'Connor, F.B., 1955. Extraction of enchytraeid worms from a coniferous forest soil. Nature 175, 815–816.
- Parkinson, J.A., Allen, S.E., 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Communications in Soil Science and Plant Analysis 6, 1–11.
- Ron Vaz, L., Edwards, T., Shand, C., Cresser, M., 1993. Quantification of phosphorus fractions in soil solution. The Science of the Total Environment 135, 67–71.
- Setälä, H., Martikainen, E., Tyynismaa, M., Huhta, V., 1990. Effects of soil fauna on leaching of nitrogen and phosphorus from experimental systems simulating coniferous forest floor. Biol. Fertil. Soils 10, 170–177.
- Setälä, H., Tyynismaa, M., Martikainen, E., Huhta, V., 1991. Mineralization of C, N, and P in relation to decomposer community structure in coniferous forest soil. Pedobiologia 35, 285–296.
- Sparling, G.P., Ord, B.G., Vaughan, D., 1981. Microbial biomass and activity in soils amended with glucose. Soil Biol. Biochem. 13, 99–104.
- Springett, J.A., Brittain, J.E., Springett, B.P., 1970. Vertical movement of Enchytraeidae (Oligochaeta) in moorland soils. Oikos 21, 16–21.
- Tabatabai, M.A., Bremer, J.M., 1969. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.
- Williams, B.L., Griffiths, B.S., 1989. Enhanced nutrient mineralisation and leaching from decomposing sitka spruce litter by enchytraeid worms. Soil Biol. Biochem. 21, 183–188.