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Soil Biology & Biochemistry 38 (2006) 1812–1822

Soil Biology & Biochemistry

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Behaviour of the endocrine disrupting chemical nonylphenol in soil: Assessing the risk associated with spreading contaminated waste to land

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Received 4 July 2005; received in revised form 18 December 2005; accepted 19 December 2005
Available online 20 February 2006

Abstract

There is increasing environmental concern about the impact of endocrine disrupting chemicals (EDCs) on ecosystem sustainability and human health. Many EDCs are present within wastes which are routinely spread to land (e.g. biosolids). The aim of this study was to investigate the behaviour and fate of the EDC, 4-nonylphenol (NP), in a range of soils and to assess the potential risk it may pose to soil and freshwaters environments. We showed that NP was not persistent in soil, that NP mineralization was concentration-dependent and was stimulated by the addition of organic residues (e.g. biosolids, glucose, dead roots) but not by the presence of a rhizosphere. NP had no negative effect on soil respiration or plant growth unless present at extreme concentrations ($\geqslant 10{,}000\,\mathrm{mg\,NP\,kg^{-1}}$) and the uptake of NP by plants was very low. While NP was sorbed to the solid phase it could easily be leached from soil. Taking all of our results together, we conclude that the spreading of NP contaminated waste soil to soil probably poses a very low environmental risk to freshwater ecosystems and human health.
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Keywords: Alkylphenol polyethoxylate; Bioremediation; Biodegradation; Ecotoxicology; Hormone; Phytoremediation; Sewage sludge

1. Introduction

A wide variety of natural and anthropogenic compounds frequently used in domestic, industrial and agricultural processes have been found to mimic reproductive hormone activities in a diverse range of organisms (Tyler et al., 1998; Nimrod and Benson, 1996; Vos et al., 2000; Wang et al., 2004; Zala and Penn, 2004). Further, many chemical pollutants known to interfere with hormones and other physiological mechanisms have become ubiquitous in the environment. It is now clear that these endocrine disrupting chemicals (EDCs) not only have harmful effects on an organism's ability to reproduce but they also affect a wide range of behaviours, including activity, motivation, communication, aggression, learning and other cognitive abilities (Zala and Penn, 2004). The emission of EDCs into the environment can severely affect specific population numbers possibly threatening the long-term viability of ecosystems (Vos et al., 2000; Brown et al., 2003). Most of the reported effects of EDCs are associated with freshwater organisms where a direct causal link can often be made between EDC presence and organism dysfunction. Research has shown that EDCs can reach watercourses by direct entry (e.g. discharge of effluents from sewage treatment works or paper processing factories; Lee et al., 2004) or indirectly (e.g. typically from farm runoff when agricultural or industrial wastes have been applied; Wang et al., 2004).

One of the most widely recognized and common anthropogenically derived EDCs present in the environment is nonylphenol (NP; Kuruto–Niwa et al., 2005). NP is a degradation product of a group of non-ionic surfactants known as alkylphenol polyethoxylates which are a common component of domestic and industrial cleaning products. Exposure of organisms to NP results in the abnormal secretion and production of the female phospholipoglycoprotein vitellogenin which is a precursor to egg yolk. Several studies have also reported the NP-induced expression of this protein in male fish, however, the long-term effects of these elevated vitellogenin concentrations remains unclear although it may reduce semen

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0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.12.006

quality (White et al., 1994; Jobling et al., 1998; Matozzo and Marin 2005; Lahnsteiner et al., 2005; Li and Wang, 2005). Although NP has been found in human foodstuffs, the long-term effects of NP on the human population are inconclusive although short-term effects have been documented (Vivacqua et al., 2003; Pflieger–Bruss et al., 2004).

NP is a pollutant that is found in significant concentrations in wastewater and anaerobically digested sewage sludge and effluent (Ahel et al., 1994). The direct application of sewage sludge and wastewater to agricultural land is a common practice and it may therefore pose a possible environmental hazard. NP is only slightly soluble in water (maximum solubility $4.9 \pm 0.4\,\mathrm{mg\,l^{-1}}$) and this can be expected to significantly affect its bioavailability and leaching potential (Brix et al., 2001). Previous studies have indicated that NP degrades rapidly in sludge-amended soil and that mineralization is a biotic process that is affected by temperature (Marcromini et al., 1989; Topp and Starratt, 2000). Recent work has confirmed that white rot fungi and *Sphingomonas* possess the ability to degrade NP (Soares et al., 2005; Corvini et al., 2004). Further information, however, is still required on the resilience of the soil microbial community to NP and the factors that regulate NP bioavailability in soil.

There have been few investigations into the toxicity and uptake of NP into plants grown in soil although its presence in foodstuffs would suggest that uptake can occur (Vivacqua et al., 2003). The presence of plant roots in soil is known to stimulate the microbial community and has been shown in some cases to stimulate the biodegradation and removal of organic pollutants. The impact of plants on NP behaviour in soil remains poorly understood.

Upon entering soil, NP can undergo a number of reactions (e.g. sorption, biodegradation, leaching, plant uptake) that ultimately control its fate and potential environmental hazard. The relative importance of these factors, however, remains poorly understood. The aim of this study was therefore to investigate the dose-dependent effects of NP on soil microbial activity in a range of soils and to investigate its phytotoxicity and the potential of plants or microbes to remediate NP from soil. Further, we assessed the sorption potential of NP in soil and the effect this has on its leaching potential. Based upon these findings, we assessed the likely risk to freshwater and humans associated with spreading NP contaminated waste to land.

2. Materials and methods

2.1. Chemicals

[*ring*-U-^{14}C] 4-nonylphenol {$^{14}C_9H_4[C(CH_2)_8CH_3]OH$)} was obtained from American Radiolabeled Chemicals Inc. (ARC Inc., St. Louis, MO; ARC900, 99% radioactive purity; specific activity of 1.85–2.22 $\mathrm{GBq\,mmol^{-1}}$). Technical grade 4-nonylphenol was obtained from Sigma (Sigma-Aldrich Co., St. Louis, MO). A stock solution of $1000\,\mathrm{mg\,l^{-1}}$ NP was made in hexane and spiked with ^{14}C–NP ($3\,\mathrm{MBq\,l^{-1}}$).

2.2. Soils and sewage sludge

Four soils were sampled in North Wales, UK. Soils 1 and 2 were collected from sheep-grazed-dominated grasslands at the Henfaes Experimental Station, Abergwyngregyn (53°14′N 4°01′W). Soil A (Eutric cambisol) is a lowland (15 m altitude) freely draining, heavily sheep-grazed grassland which receives regular fertilization (120 kg N, 60 kg K and $10\,\mathrm{kg\,P\,yr^{-1}}$) and supports a sward consisting predominantly of perennial ryegrass (*Lolium perenne* L.), clover (*Trifolium repens* L.) and crested dog's tail (*Cynosurus cristatus* L.). Soil B (Haplic podzol) was collected from an upland (200 m altitude) freely draining, heavily leached, lightly sheep-grazed grassland which receives no fertilization and supports a grassland sward consisting predominantly of sheep's fescue (*Festuca ovina* L. var. Ovina) and common bentgrass (*Agrostis capillaris* L.). The mean annual soil surface temperature at 10 cm varies from 8 °C to 10 °C and the annual rainfall at the lowland site is 1250 mm and at the upland site 1700 mm.Soil C was collected from Aberffraw, Anglesey (53°11′N 4°27′W) and is a lowland (10 m altitude) sandy textured Eutric cambisol which is lightly grazed by sheep, receives no fertilization and supports a grassland sward consisting predominantly of perennial ryegrass. Soil D was collected from Rhôs (53°18′N 3°45′W) and is a lowland (altitude 15 m) ungrazed Eutric cambisol supporting perennial ryegrass which is mown regularly. Samples were collected from different depths in the soil profile by digging soil pits approximately 0.5 m × 0.7 m wide and 0.6 m deep. Soil was then collected from three of the exposed soil profile faces (Table 1).

Biosolids (SS) were obtained from a large municipal wastewater treatment plant located in Treborth, Bangor, North Wales (Dŵr Cymru Ltd., Treharris, Mid Glamorgan, Wales). This is dewatered tertiary treated sludge and is derived mainly from domestic wastewater with small contributions from light industry.

pH was determined in a 1:2.5 (w/w) solid:water extract while moisture content was determined by drying at 105 °C. Organic matter was determined by ashing at 450 °C overnight. Exchangeable cations were removed from soil/biosolids by performing a 1:10 (w/v) soil:0.5 M $BaCl_2$ extract for 1 h. The extracts were then centrifuged (10 min, 13,000*g*) and the supernatant recovered for analysis using an Ultrace ICP-OES (Horiba-Jobin Yvon SAS, Longjumeau, France). Available P was determined by performing a 1:10 (w/v) soil:0.5 M acetic acid extract for 1 h and determination of P in the extract colorimetrically (Murphy and Riley, 1962).

2.3. Phytotoxicity of NP

Known concentrations of NP dissolved in hexane were added to 10 g of acid-washed fine quartz sand and the

Table 1
General characteristics of the soils used in the NP behaviour experiments

Soil	Texture	pH	Organic matter (%)	Exchangeable cations (mmol kg^{-1})						P (mg kg^{-1})
				Al	Ca	Na	K	Mg	Mn	
A	Clay loam	6.18	2.91	0.6	12.5	1.0	4.1	1.6	0.14	47.5
B	Sandy loam	4.50	8.9	9.8	2.5	1.0	1.4	0.7	0.12	0.1
C	Sand	5.58	7.96	3.6	13.2	0.6	1.5	1.7	0.08	8.5
D	Sandy silt loam	6.74	5.61	0.2	46.7	0.8	4.6	1.9	0.02	20.4

Only results for the 0-15 cm soil layer are presented. The values represent means ($n = 3$).

hexane allowed to evaporate overnight. The NP-coated sand (10 g) was then lightly shaken with 40 g of field moist soil or biosolids to give uniform soil coverage and NP concentrations of 0 (control), 10, 100, 1000 and 10,000 mg NP kg^{-1}. Polypropylene tubes were then either filled with 40 g of the soil/sand mix to a bulk density of 0.9 kg dm^{-3} and planted with three pre-germinated seeds of wheat (*Triticum aestivum* L. cv '*Scout 66*') or oil seed rape (*Brassica napus* L. cv '*Apex*'). These were covered with 3 g of soil, arranged in a randomized design and allowed to grow in a controlled-environment chamber with day/night rhythm of 22/18 °C, relative humidity of 51%, 12 h photoperiod and light intensity of 250 μmol m^{-2} s^{-1}. This temperature reflected summer growth conditions at the study sites. The plants were watered daily to maintain a gravimetric moisture content between 20% and 25%. Shoot height was recorded weekly and plants were harvested after 14 d and shoots and root biomass recorded after drying (40 °C, 48 h).

In an additional experiment, uncontaminated soil was mixed with NP contaminated biosolids on a 1:1 (w/w) basis and a phytotoxicity trial performed as described above.

2.4. Effect of NP on microbial respiration

Soil contaminated with different amounts of NP (1–10,000 mg NP kg^{-1}) was prepared as described above. Basal soil respiration was then measured over a 14-d period at 10 °C using an automated MSR-1 multi-channel soil respirometer with CIRAS-SC gas analyser (PP Systems Ltd., Hitchin, UK).

2.5. Microbial degradation of NP

Soil from different depths were contaminated with varying amounts of ^{14}C-labelled NP (1–10,000 mg NP kg^{-1}) as described above and placed in 40 cm^3 glass containers. A polypropylene vial containing 1 ml of 1 M NaOH was then placed into each soil container to catch evolved $^{14}CO_2$. The containers were then hermetically sealed and placed in a 10 °C incubator. This temperature was chosen to reflect the mean annual soil temperature of the sample sites. The $^{14}CO_2$ traps were replaced after 1, 3, 5 and 10 d and every 10 d thereafter up to 60 d. The amount of $^{14}CO_2$ captured in the NaOH traps was determined using a Wallac1409 liquid scintillation counter (Wallac EG&G Ltd., Milton Keynes, UK) and OptiPhase 3 scintillation cocktail (Wallac EG&G Ltd.). A Michaelis–Menten kinetic model was fitted to the experimental data where

$$V = V_{max} \times C/(C + K_m)$$

where V is the rate of NP mineralization, V_{max} is the maximum rate of NP mineralization, C is the soil NP concentration and K_m is the affinity constant.

In addition, ^{14}C-labelled NP sand was mixed with a 1:1 (w/w) mixture of soil and biosolids to give NP concentrations ranging from 10 to 1000 mg kg^{-1}. The rate of $^{14}CO_2$ evolution measured as described above over a 100-d period (+biosolids). Comparisons were made to soil in which the ^{14}C-labelled NP sand had been added directly to the soil (-biosolids).

In addition, soils A and C were contaminated with ^{14}C–NP (1000 mg NP kg^{-1}) as described above and a range of additives were then mixed in with the soils to determine whether they influenced the rate of NP biodegradation. The treatments included (1) the addition of labile C and N substrates to the soil (10.0 mg g^{-1} of glucose and 1.0 mg g^{-1} of glutamate to simulate root exudation, (2) the addition of NPK fertilizer to the soil at a rate of 1 mg N g^{-1}, 0.1 mg P g^{-1} and 0.1 mg K g^{-1}, (3) a combination of treatments 1 and 2, (4) the addition of plant residues in the form of excised maize roots at a rate of 20 mg FW g^{-1} to simulate root turnover and, (5) unamended soil (control). The soil was placed in tubes and $^{14}CO_2$ evolution monitored as described above.

2.6. NP sorption to soil

An aqueous solution containing ^{14}C-labelled NP at maximum solubility (10 ml; 5 mg NP l^{-1}) was added to 0.5 g of each field moist soil contained in glass tubes. The tubes were then shaken at 200 rpm for 30, 60, 90, and 120 min. After shaking, the tubes were centrifuged (14,000*g*, 5 min) and the ^{14}C–NP remaining in the supernatant determined by liquid scintillation counting as described above. The amount of NP sorbed to the solid phase was calculated as

the difference between the initial and final NP solution concentration.

2.7. NP leaching from soil

Soil contaminated with ^{14}C-labelled NP (1000 mg NP kg^{-1}; 336 kBq kg^{-1}) was prepared as described above and 3 g placed in glass leaching columns (10 cm long × 1 cm diameter). Distilled water was then added to the columns at a rate of 0.2 ml min^{-1} for 2 h and the eluent from the columns collected over time in polypropylene scintillation vials. The amount of ^{14}C–NP in the leachate was determined by liquid scintillation counting as described above.

2.8. NP rhizoremediation

Soils A and B were contaminated with ^{14}C-labelled NP (1000 mg NP kg^{-1}) as described above. Each soil (65 g) was then placed into 50 ml polypropylene tubes and planted with pre-germinated maize. To allow the capture of any $^{14}CO_2$ evolved, the planted tubes were placed into larger glass vessels through which air could be continually passed. This prevented re-fixation of CO_2 by plants. The air leaving the glass vessels was subsequently passed through 1 M NaOH to trap $^{14}CO_2$. The NaOH traps were replaced every 3 d and the amount of ^{14}C within them (NP_{resp}) determined by liquid scintillation counting as described previously. Plants were grown in a climate-controlled chamber (see above for details) for 21 d, after which time the microcosms were destructively harvested. Roots were carefully separated from the soil and washed free of soil with distilled water. Both shoots and roots were dried at 80 °C overnight and their ^{14}C content (NP_{root}, NP_{shoot}) determined with a Biological Oxidizer OX400 (RJ Harvey Instrument Corp., Hillsdale, NJ). Soil from the microcosms was extracted with methanol (1:5 w/v; 30 min, 225 rpm), the extract centrifuged (10 min, 6000*g*) and the ^{14}C in the supernatant (NP_{ext}) determined as described above. The amount of ^{14}C remaining in the soil (NP_{soil}) was calculated by difference where

$$NP_{soil} = NP_{tot} - NP_{shoot} - NP_{root} - NP_{ext} - N_{resp}$$

where NP_{tot} was the total amount of ^{14}C–NP added to the microcosms. Unplanted microcosms containing ^{14}C–NP were used as a control.

2.9. Statistical analysis

All treatments were carried out in triplicate. Statistical analyses (*t*-tests, linear regression, ANOVA) were performed using Sigmaplot 4.01 (SPSS Inc., Chicago, IL) and Minitab 14 (Minitab Inc., State College, PA). Fitting of the Michaelis–Menten kinetic model and the double exponential kinetic model to the experimental mineralization data was made using Sigmaplot 4.01.

3. Results

3.1. Phytotoxicity of NP

Based upon plant biomass production, wheat tended to be more tolerant to NP than oil seed rape (Fig. 1). Apart from a reduction in the overall growth of roots and shoots, there were few other obvious visual symptoms of NP toxicity in either plant species although necrotic regions in the leaves of shoots were more abundant at the highest NP concentration. It is not clear whether these necrotic lesions were a direct consequence of NP exposure or an indirect effect of poor plant health and reduced resistance to disease. A significant reduction in wheat growth was only observed at the highest soil NP concentration in all the test soils (10,000 mg kg^{-1}; $P<0.05$). In contrast to wheat, oil seed rape growth was significantly affected in three of the test soils at an NP concentration of 1000 mg kg^{-1}. In the fourth soil, the growth of oil seed rape was not significantly repressed until grown in the presence of 10,000 mg NP kg^{-1}. The concentration at which NP caused

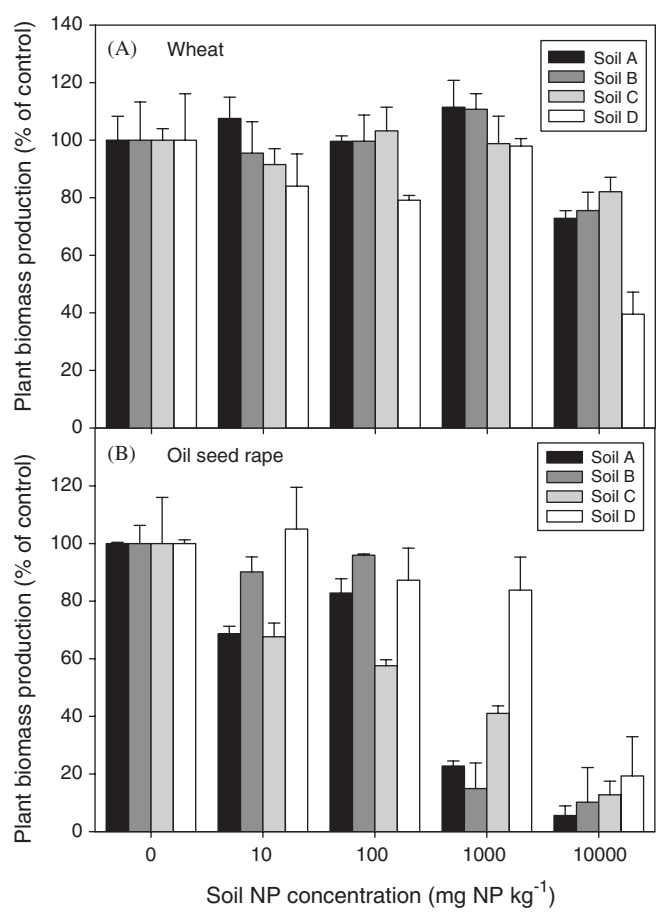

Fig. 1. Effect of increasing concentrations of the EDC, NP (1–10,000 mg kg^{-1} soil) on the growth of wheat (panel A) and oil seed rape (panel B) in four agricultural grassland soils. Values represent means ± SEM ($n = 3$).

a 50% reduction in plant growth (LC_{50}) are summarized in Table 2 for the four test soils.

The presence of NP-contaminated biosolids in the soil had no significant effect on plant growth in any of the soils examined at any of the added NP concentrations examined (10–1000 mg NP kg^{-1}; $P>0.05$; data not presented).

3.2. Soil microbial activity

In the absence of NP, the basal soil respiration from the four experimental sites differed markedly from each other ($P<0.05$; data not presented), however, they all showed a similar response pattern upon the addition of NP up to concentrations of 1000 mg NP kg^{-1}. Consequently, only the results for Soil A are presented (Fig. 2). In general, low rates of NP addition to the soil (1–1000 mg kg^{-1}) caused a small but significant increase in soil respiration which persisted for approximately 7 d ($P<0.05$). In contrast, the addition of NP at concentrations of 10,000 mg kg^{-1} resulted in a 50–80% reduction in soil respiration in three of the soils ($P<0.05$), while in the fourth soil the rate of respiration was not significantly different from the unamended (control) soil ($P>0.05$). To investigate whether NP caused a priming of soil organic matter turnover, we calculated the amount of C evolved as CO_2 over the 14 d experimental period expressed as a percentage of the total C added in the NP. The results indicated that at low NP addition rates (1 and 10 mg NP kg^{-1}), more C was recovered as CO_2 than the amount of C added in the NP. This increase in the turnover of native soil organic matter was equivalent to a 3–9% increase in basal soil respiration. At higher NP addition rates (100 and 1000 mg NP kg^{-1}), the amount of C recovered as CO_2 was much less than the amount added in the NP (8–40% of the C in NP recovered as CO_2). In this case, the amount of C recovery from the added NP was slightly higher than predicted to occur after 14 d using ^{14}C-labelled NP (Section 3.3) again providing an indication of SOM priming.

Table 2
Toxicity of the EDC, NP, expressed as LC_{50} values for wheat and oil seed rape after growth in four contrasting soils amended with increasing concentrations of NP

	LC_{50} value (mg NP kg^{-1} soil)			
	Soil A	Soil B	Soil C	Soil D
Wheat	ND	ND	ND	9500
Oil seed rape	800	650	1000	3500

Values represent means ($n=3$). LC_{50} is defined as the concentration at which plant biomass was reduced by 50% relative to that in unamended soil. ND indicates that the LC_{50} value was greater than 10,000 mg NP kg^{-1} soil.

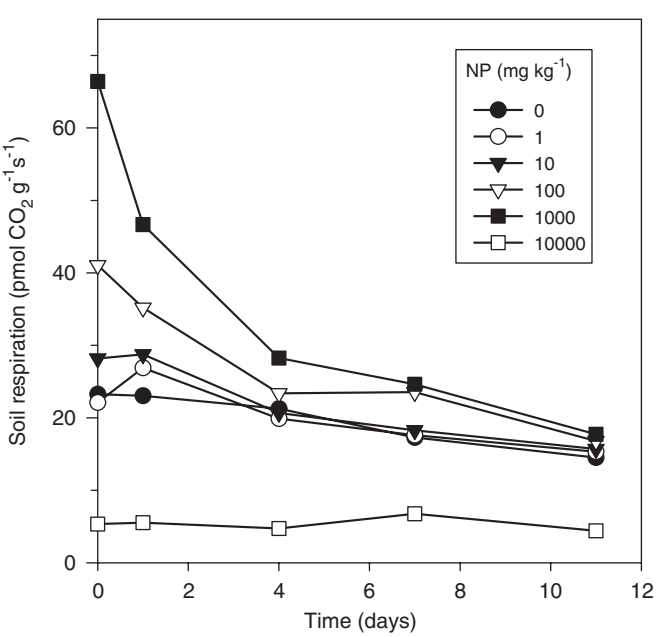

Fig. 2. Effect of increasing concentrations of the EDC, NP (1–10,000 mg kg^{-1} soil) on basal soil respiration in Soil A. Values represent means ± SEM ($n=3$).

3.3. Microbial degradation of NP

Generally, the biodegradation of NP in soil showed an initial phase of rapid mineralization within the first 10 d, followed by a slower secondary mineralization phase (Fig. 3). This $^{14}CO_2$ evolution pattern was particularly apparent at NP addition rates $\leqslant 100$ mg kg^{-1}. In all soils, the rate of mineralization was most rapid in the upper soil layers and tended to decline with soil depth. The amount of NP biodegradation as a proportion of the total NP added to the soil decreased with NP concentration (Fig. 3). However, when the rates of NP mineralization were calculated (mg NP g^{-1} h^{-1}) it was apparent that in all soils the greatest degradation rate occurred at the highest added NP concentrations (Fig. 4). From a limited number of data points it appeared that NP mineralization exhibited Michaelis–Menten-type kinetics ($r^2=0.85-0.99$). From the Michaelis–Menten equation, the maximum rate of NP mineralization (V_{max}) in Soil A was predicted to decrease down the soil profile being 13, 2.4, 1.7 and 1.5 mg NP kg^{-1} h^{-1} in the four successive soil layers, respectively (0–15, 15–30, 30–45, 45–60 cm). The results for the other three soils were similar (data not presented). The affinity constant (K_m) values showed no obvious pattern with soil depth giving values ranging from 20 to 250 mg NP kg^{-1} (mean ± SEM = 133 ± 50 mg NP kg^{-1}).

The addition of biosolids to the soil significantly increased the rate of NP mineralization throughout the 100 d incubation period in comparison to those to which no biosolids were added ($P<0.05$). The relative biosolids-induced increase in NP mineralization was soil dependent, increasing 5-fold in Soil B and 1.4-fold in Soils A and C. Soil D was not tested in this experiment (Fig. 5). These

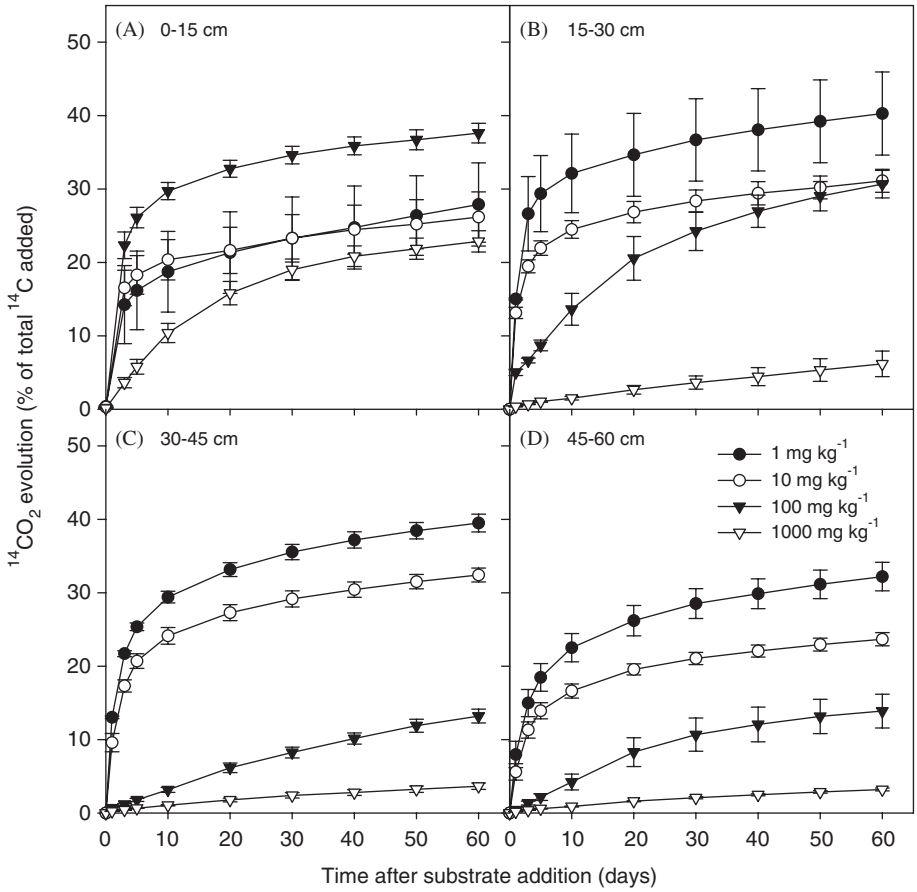

Fig. 3. Time-dependent mineralization of different concentrations of ^{14}C-labelled NP (1–1000 mg kg^{-1}) after addition to different depths of soil A. Panel A shows the 0–15 cm layer, panel B the 15–30 cm layer, panel C the 30–45 cm layer, and panel D the 45–60 cm layer. Values represent means ± SEM ($n = 3$).

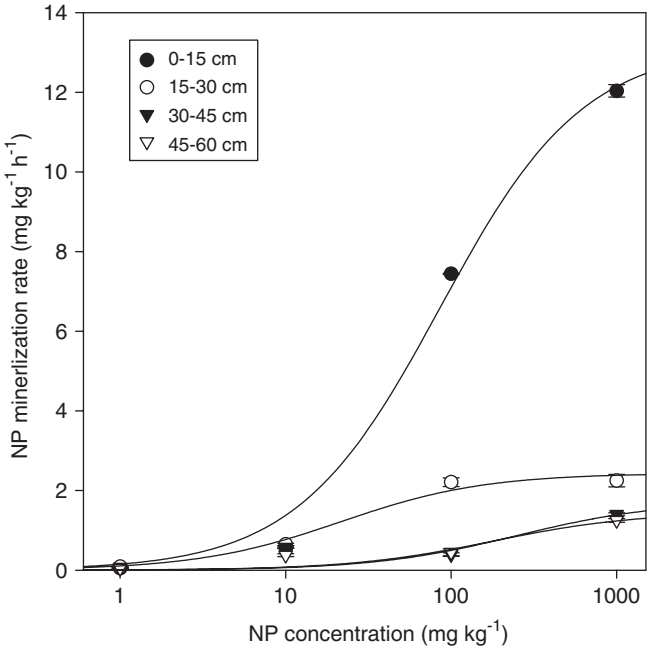

Fig. 4. Concentration-dependent mineralization kinetics of ^{14}C-labelled NP in Soil A. Symbols represent experimental data points while curves represent fits of a Michaelis–Menten kinetic equation to the experimental data. Values represent means ± SEM ($n = 3$).

increases in mineralization were consistent throughout the 100 d incubation period (data not presented).

Amending the soil with either labile C substrates (to simulate root exudation; Exudates) or NPK fertilizer caused an initial suppression of NP mineralization in all soils in comparison to the control soil (Fig. 6). In contrast, the addition of excised roots to the soil (to simulate root turnover; Roots) caused a significant increase in the rate of NP mineralization ($P < 0.05$). After 20 d, however, the rate of NP mineralization in the exudates + NPK treatment increased significantly and after 45 d the amount of mineralization was greater than in all the other treatments ($P < 0.05$). At the end of the experiment (120 d) the amount of NP mineralized in all the amendment treatments (NPK, exudate and root addition) was significantly greater than observed in the control soil ($P < 0.05$) and followed the trend:

NPK + exudates > NPK > Exudates = Roots > control.

3.4. Influence of plants on NP mineralization

The presence of plants and their associated rhizosphere had no significant impact on the rate of NP mineralization in Soil A over a 3-week period ($P > 0.05$; Fig. 7). In

contrast, although the temporal pattern of NP mineralization in Soil B was the same in the unplanted and planted microcosms, the rate of mineralization was significant lower in the presence of plants ($P<0.05$). At the end of the experiment, only small amounts of the ^{14}C label was recovered in the plants (<5% of the total ^{14}C–NP added). Of that recovered, most was present in the roots (81±2%) relative to the shoots (19±2%). We did not identify the chemical form of the ^{14}C in the plants. Consequently, the ^{14}C may represent transformation products of NP produced within the plants or released by the microbial community and taken up by the roots rather than NP itself. At the end of the 21 d period <20% of the total ^{14}C label could be recovered in a methanol extract from the soil suggesting that most of the ^{14}C had become immobilized.

3.5. Solid phase sorption of NP

Sorption of NP to the soil solid phase was a rapid process with the solid-to-solution equilibrium for NP reached within 30 min (Fig. 8). Generally, the temporal

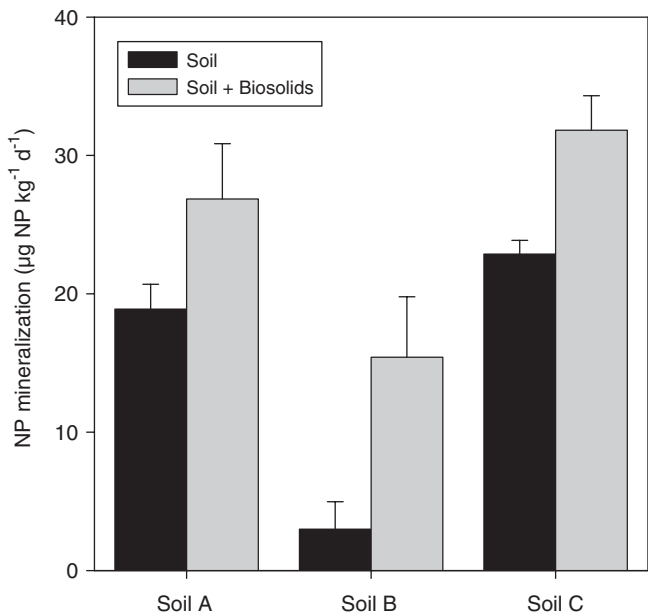

Fig. 5. Effect of biosolids on the mineralization of ^{14}C-labelled NP in three soils after 7 d. The initial NP concentration was 1 mg kg^{-1}. Values represent means±SEM ($n=3$).

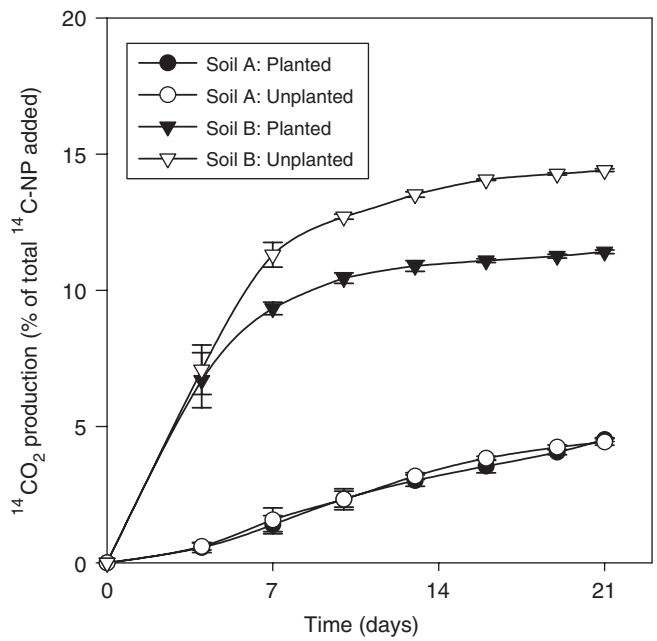

Fig. 7. Effect of the presence of maize plants on the mineralization of ^{14}C-labelled NP in soil. Values represent means±SEM ($n=3$).

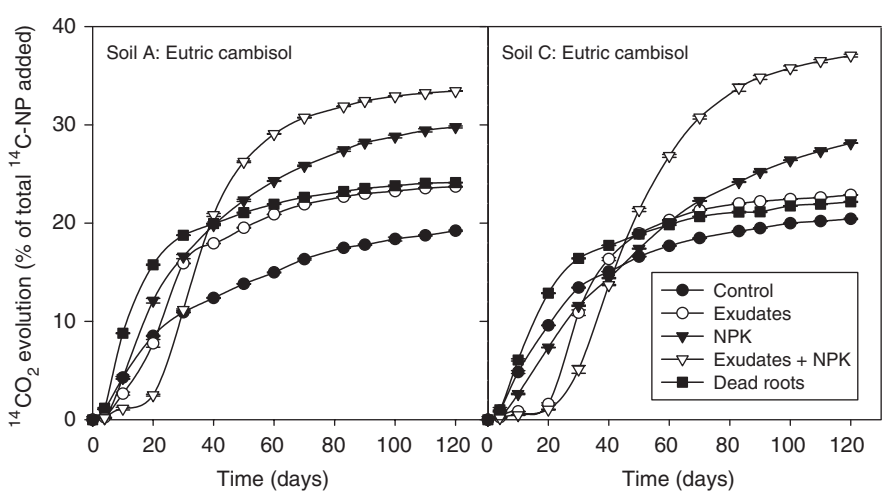

Fig. 6. Mineralization of ^{14}C-labelled NP after the amendment of two agricultural grassland soils with either (1) artificial root exudates (glucose and glutamate), (2) inorganic nutrients in the form of NPK fertilizer, (3) artificial exudates and NPK fertilizer together, (4) dead roots of maize. An unamended soil was used as the control. Values represent means±SEM ($n=3$). The legend is the same for both panels.

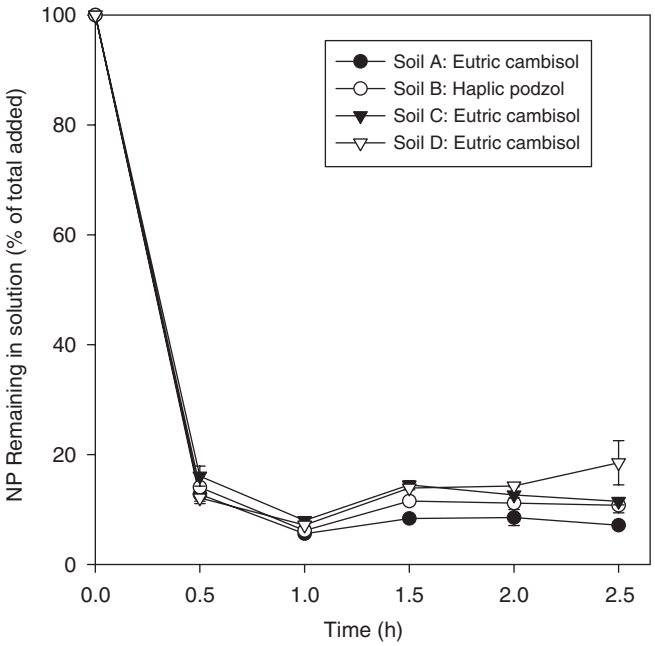

Fig. 8. Time-dependent sorption of ^{14}C-labelled NP to four contrasting soils. The initial concentration of NP in solution was $5\,mg\,l^{-1}$. Values represent means ± SEM ($n = 3$).

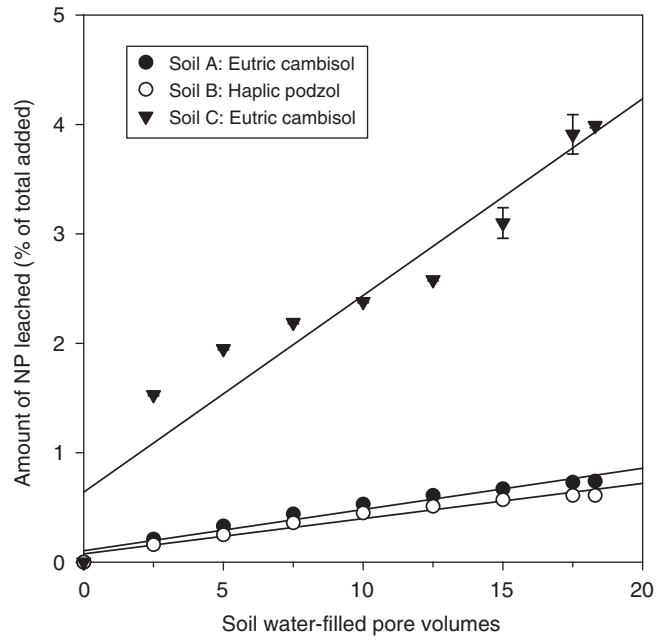

Fig. 9. Leaching of ^{14}C-labelled NP from three contrasting soils after the addition of water to the surface of the soil columns uniformly contaminated with NP at a concentration of $1000\,mg\,NP\,kg^{-1}$ soil. Soil pore volume is defined as the total water-filled pore space in the soil at saturation. Values represent means ± SEM ($n = 3$).

patterns of NP sorption were the same for the four soils tested ($P > 0.05$). After 2.5 h, approximately 85% of the added NP had become sorbed to the solid phase. The solid-to-solution partition coefficient (buffer power; Barber, 1995) was calculated to range from 80 to 120 depending upon soil type. These values are similar to those reported previously for NP (During et al., 2002).

3.6. Leaching of NP from soil

The rapid leaching of NP from soil in response to the addition of water, at a loading rate equivalent to a large storm or irrigation event, is shown in Fig. 9. The leaching potential was soil dependent and was maximal in the sandy textured soil in comparison to the loamy soils. After the first eluant fraction had been collected from the leaching columns, the rate of NP leaching showed a linear pattern over the 2-h period. In the sandy soil, the concentration of NP in the leachate was $5.0\,mg\,l^{-1}$ which is similar to the maximum solubility of NP in water.

4. Discussion

4.1. Toxicity of NP to plants

The growth trials undertaken here with four soils and two plant species indicated that plant biomass production was not significantly affected by the presence of NP in soil or biosolids unless NP was present at extremely high concentrations ($> 1000\,mg\,NP\,kg^{-1}$). The LC_{50} values for the two plant species, however, were much higher than the reported maximum environmental concentration either in soils or biosolids (typically $< 25\,mg\,kg^{-1}$ in soil and 5–$1000\,mg\,kg^{-1}$ in biosolids; Bennie, 1999; Environment Canada, 2000; Widarto et al., 2004). Based upon the maximum solubility of NP in water ($5\,mg\,l^{-1}$) and our calculated solid-to-solution partition ratio of 100, we calculate that this maximum concentration of NP in solution equates to a total soil concentration of $\leqslant 500\,mg\,NP\,kg^{-1}$. As plant growth was not affected at these NP concentrations we conclude that NP in the aqueous phase is not-phytotoxic. Phytotoxicity observed at higher NP concentrations ($> 1000\,mg\,kg^{-1}$) is likely to be due to the presence of a large amounts of light non-aqueous phase NP (LNAPL) in the soil. If roots encounter pockets of LNAPL in the soil it can be expected to directly affect root membrane integrity. In addition, our evidence suggests that large amount of LNAPL in the soil ($> 1000\,mg\,NP\,kg^{-1}$) inhibits microbial activity which may also indirectly affect plant growth by inhibiting nutrient supply and the formation of symbiotic associations.

4.2. Toxicity of NP to soil organisms

The LC_{50} concentrations reported here for NP phytotoxicity are similar to those for some terrestrial mammals when taken orally (rats and mice $LD_{50} = 1300$–$2600\,mg\,kg^{-1}$; de Jager et al., 2001). Based upon the high

no-observed-adverse-effect-level (NOAEL) for uptake of NP by small mammals ($15\,mg\,kg^{-1}\,d^{-1}$; APERC, 2002), it is unlikely that the incorporation of NP biosolids into soil will pose a significant environmental risk to mammals living in soil. With regard to soil-dwelling organisms such as earthworms and collembola, the toxicological effects of NP may be expected to be exacerbated due to direct ingestion. However, studies have shown that these geophagous organisms are relatively insensitive to the addition of NP-contaminated biosolids to soil with few effects observed at concentrations $<50\,mg\,NP\,kg^{-1}$ soil (APERC, 2002; Widarto et al., 2004). Our study also indicates that soil microorganisms are not adversely affected at low soil NP concentrations ($<1000\,mg\,NP\,kg^{-1}$). Taking all of our results together with those of others it would appear that the amount of NP expected to be present in soil after the landspreading of biosolids will have few deleterious effects on the plant–soil system. This view is reinforced by the widespread enhancement of agricultural production in fields which have repeatedly received biosolids particularly those with low heavy metal content.

4.3. Loss of NP from soil to freshwater

In contrast to higher plants and soil organisms which are relatively insensitive to NP, freshwater organisms (e.g. crustaceans, fish, insects, nematodes, zooplankton etc.) are extremely sensitive and low-level exposure can cause severe reproductive disorders ($<0.1\,\mu g\,l^{-1}$) and even death ($LC_{50} = 0.1 - 5\,mg\,l^{-1}$; Ekelund et al., 1990; Liber et al., 1999). Aquatic plants are also more susceptible to NP than those grown in soil ($NOAEL = 0.2\,\mu g\,l^{-1}$; Liber et al., 1999). While biosolids application to land may pose little direct danger, there is an apparent risk that runoff and leachate from soils will contaminate surface waters where the consequences may be more severe. Although runoff and leaching of biosolids components have been well documented (e.g. P, NO_3^-; Penn and Sims, 2002) the leaching of NP from soil remains less studied. Our results clearly show that leaching of NP is clearly possible and that concentrations may be significant at high ($5\,mg\,l^{-1}$) soil loading rates. Typically, however, it is recommended that sludge be spread at least 15 m from adjacent watercourses to minimize the risk of pollution (NSEL, 2004). Based upon the loss of other solutes present in biosolids it is likely that under normal landspreading activities the amount of NP lost to watercourses will be very low (Penn and Sims, 2002; Jacobsen et al., 2004; Richards et al., 2004). Even under exceptional circumstances where runoff does occur (e.g. during storm events, flooding, soil erosion) it is likely that these will be isolated incidents where the dilution in the water will be very large. This is reinforced by Environment Agency UK reports which show many pollution events occurring from poor control of discharges from sewage treatment works but almost none due to the inappropriate management of biosolids.

4.4. Microbial degradation of NP

Our study showed that the mineralization of NP in soil was initially very rapid particularly at low concentrations after which the rate of mineralization declined. Extracts of the soil at this point indicated that although NP mineralization was incomplete, very little of the added ^{14}C–NP could be recovered from the soil during extraction with methanol (in which NP is soluble). We conclude that at this point the ^{14}C was either no longer bioavailable, or more likely, had been utilized and transformed into other products by the microbial biomass. The two-phase pattern of $^{14}CO_2$ evolution is similar to many low-molecular weight substrates in soil in which the initial fast phase of mineralization reflects the immediate use of the substrate in catabolic processes while the secondary phase reflects the turnover of the microbial biomass after the anabolic processing of the substrate (Jones et al., 2004). The mineralization of substrates by this model can subsequently be described by a double first-order kinetics model. Analysis of the results presented in Fig. 3 for the 0–15 cm layer indicated an extremely close fit to the kinetic model ($r^2 = 0.998-0.999$) giving half-life values ranging from 1.4 to 10.6 d for NP in soil. Similarly, the other underlying soil layers also gave close fits to the model ($r^2 = 0.992-0.999$) and NP half-lives ranging from 1–3 d at low NP concentrations ($1–10\,mg\,kg^{-1}$) to 10–42 d at higher NP concentrations ($100–1000\,mg\,kg^{-1}$). These relatively short half-lives in soil are supported by the respiration data in Fig. 2 which also showed that the NP-induced increase in soil respiration was short lived lasting for 1–4 d at low NP concentrations ($1–10\,mg\,kg^{-1}$) and up 10 d at higher NP concentrations ($100–1000\,mg\,kg^{-1}$). Taken together our results suggest that NP is not persistent in soil and consequently the window of opportunity for passage of NP from soil to freshwater is very small (i.e. within 7 d of application to land). Similarly, the chances of significant accumulation of NP in plants to a point where human toxicity is invoked is also unlikely.

Previous studies have shown that the presence of plants and its associated rhizosphere enhances the degradation of organic and xenobiotic compounds in soil (Hsu and Bartha, 1979). Our results, however, showed that plants had relatively little effect on the rate of NP mineralization in soil. This is in agreement with the results of Bokern et al. (1998) where transformation but no mineralization of NP occurred within the plant. In contrast, the addition of large amounts of nutrients or C in the form of dead roots, low molecular weight substrates, or biosolids accelerated NP mineralization in soil. It has been suggested that the addition of either sugars or nutrients can retard the mineralization of complex substrates such as xenobiotics (Manilal and Alexander, 1991; Madigan et al., 2000). A similar pattern was observed in the initial stages of this study although in the later stages mineralization rates in those soils augmented with NPK overtook all others and the final result was the removal of substantially increased

levels of ^{14}C–NP. In both soils, the combination treatment resulted in the removal of significantly higher levels of ^{14}C–NP removal.

4.5. Conclusions

Based upon reports in the literature, it is likely that the spreading of wastes contaminated with EDCs to land is a common phenomenon (Abad et al., 2005). The results presented here suggest that the EDC nonylphenol is rapidly biodegraded in soil and that this is stimulated by the addition of organic substrates to the soil but not by the presence of a rhizosphere. The addition of NP to soil at environmentally relevant rates appeared to have no detrimental effects on either plant growth or soil microbial activity. While NP is capable of being leached from soil, its short half-life means that its passage from soil to freshwater will be low apart from under storm events when its subsequent dilution will be high and its discharge time short. In comparison to the long-term release of NP from sewage treatment works directly into freshwaters, it is likely that the spreading of contaminated waste (e.g. biosolids) to land poses a minimal risk to the environment assuming that landspreading is done responsibly. Further, the low rates of NP uptake into plants and dilution in the food chain mean that the risk to human health is probably very low.

Acknowledgements

Thanks are due to Noriko Shiraishi for help with the experimental work. This work was financed by the European Union, European Social Fund.

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