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Soil Biology & Biochemistry 38 (2006) 3372–3379

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Saprotrophic fungi transform organic phosphorus from spruce needle litter

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Received 14 February 2006; received in revised form 5 May 2006; accepted 10 May 2006
Available online 27 June 2006

Abstract

Fungal decomposition of and phosphorus transformation from spruce litter needles (*Picea abies*) were simulated in systems containing litter needles inoculated with individual saprotrophic fungal strains and their mixtures. Fungal strains of *Setulipes androsaceus* (L.) Antonín, *Chalara longipes* (Preus) Cooke, *Ceuthospora pinastri* (Fr.) Höhn., *Mollisia minutella* (Sacc.) Rehm, *Scleroconidioma sphagnicola* Tsuneda, Currah & Thormann and an unknown strain NK11 were used as representatives of autochthonous mycoflora. Systems were incubated for 5.5 months in laboratory conditions. Fungal colonization in systems and competition among strains were assessed using the reisolation of fungi from individual needles. After incubation, needles were extracted with NaOH and extracts were analysed using ^{31}P nuclear magnetic resonance spectroscopy (NMR). Needle decomposition was determined based on the decrease in C:N ratio. Systems inoculated with the basidiomycete *S. androsaceus* revealed substantial decrease in C:N ratio (from 25.8 to 11.3) while the effect of ascomycetes on the C:N ratio was negligible. We suppose that tested strains of saprotrophic ascomycetes did not participate substantially in litter decomposition, but were directly involved in phosphorus transformation and together with *S. androsaceus* could transform orthophosphate monoesters and diesters from spruce litter needles into diphosphates, polyphosphates and phosphonates. These transformations seem to be typical for saprotrophic fungi involved in litter needle decomposition, although the proportion of individual phosphorus forms differed among studied fungal strains. Phosphonate presence in needles after fungal inoculation is of special interest because no previous investigation recorded phosphonate synthesis and accumulation by fungi. Our results confirmed that the ^{31}P NMR spectroscopy is an excellent instrumental method for studying transformations of soil organic phosphorus during plant litter decomposition. We suggest that polyphosphate production by *S. androsaceus* may contribute to the phosphorus cycle in forest ecosystems because this fungus is a frequent litter colonizer that substantially participates in decomposition.

Keywords: ^{31}P NMR; Decomposition; Organic phosphorus; Polyphosphates; Phosphonates; *Picea abies*; *Setulipes androsaceus*

1. Introduction

Fungal decomposition of coniferous litter is one of the main processes in forest ecosystems. Saprotrophic fungi responsible for litter decomposition play an important role in the cycling of nutrients including phosphorus. Phosphorus extraction and radioactive isotope tracing demonstrated the fungal ability to accumulate phosphorus in their fruit bodies (Vogt and Edmonds, 1980), transport it a substantial distance via substrate mycelia or specific mycelial structures (Wells and Boddy, 1995; Cairney, 1992) and compete for phosphorus with ectomycorrhizal fungi (Lindahl et al., 1999). As a result of these processes, fungal mycelium may contain a substantial pool of accessible organic phosphorus. However, clear evidence of the effect of individual fungal species on organic phosphorus transformation during decomposition has not yet been published. This role is usually generally accredited to soil fungi, bacteria or microorganisms (Turner et al., 2004a).

The solution ^{31}P nuclear magnetic resonance spectroscopy (NMR), presently the most precise method to

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0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.05.007

characterize phosphorus forms, is enormously helpful during studies of phosphorus transformation. ^{31}P NMR spectroscopy, frequently used to study phosphorus extracted from soils (Bedrock et al., 1994; Turner et al., 2003, 2004a, b), from plant leaves and microbial biomass (Makarov et al., 2002, 2005) and phosphorus exchange within mycorrhizal roots (see Pfeffer et al., 2001), allows to distinguish the following forms of phosphorus present in soil and organisms: organic monoesters, diesters, polyphosphates, phosphonates and inorganic orthophosphates. However, reproducible and quantitative results of phosphorus transformation in ecosystems can be obtained with ^{31}P NMR spectroscopy only when standard conditions of extraction and spectra measurement are met (Novák et al., 2005a).

In the present paper, the advantages of solution ^{31}P NMR spectroscopy are demonstrated on spruce litter needle decomposition using selected model fungal strains representing autochthonous saprotrophic mycoflora. Our study aims to: (i) reveal the effect of particular fungal strains on organic phosphorus transformation from spruce litter needles and on needle decomposition and (ii) determine the effect of fungal competition on these processes and trace effects of particular strains in the mixture variants.

2. Material and methods

2.1. Cultivating systems and fungal strains

Spruce litter was collected from the O_f horizon in a native mountain stand of *Picea abies* [L.] Karst. near "Novohut'ský močál" swamp in the Bohemian Forest (Šumava National Park, Czech Republic). Collected litter was air dried and sieved through a 2 mm sieve to obtain needles and small detritus only. Cultivating systems consisted of a glass Petri dish (18 cm in diameter) with plastic mesh (0.5 mm mesh size) placed on the bottom of the dish and covered with non-woven textile to protect water logging. Systems were filled with 15 g of air-dried needles that were slightly pressed to form approximately a 5-mm-thick compact layer. Systems were sterilized with gamma radiation. Sterile systems were moistened with enough sterile distiled water to provide the original humidity of needles (30 ml H_2O per system) and then inoculated with selected fungal strains.

Fungal strains representing the autochthonous mycoflora were isolated from needles sampled in O_f soil horizon of the same stand using the method described earlier (Koukol et al., in press). The following strains were selected for the experiment: saprotrophic basidiomycete *Setulipes* (*Marasmius*) *androsaceus* (L.) Antonín (CCBAS 859/I) and five saprotrophic ascomycetes: *Chalara longipes* (Preus) Cooke (CCF 3367), *Ceuthospora pinastri* (Fr.) Höhn. (CCF 3551), *Mollisia minutella* (Sacc.) Rehm (CCF 3550), *Scleroconidioma sphagnicola* Tsuneda, Currah and Thormann (CCF 3545) and an unknown strain NK11. The unknown strain NK11 was determined to be a sterile member of *Helotiales* based on the ITS1 rDNA sequencing and 100% alignment with the sequence of strain AY599235 available in the GenBank database of the National Center for Biotechnology Information (NCBI) using Blast software.

Systems with litter needles were inoculated in the following eight variants: systems with individual fungal strains (*S. androsaceus*, *C. longipes*, *C. pinastri*, *M. minutella*, *S. sphagnicola* and NK11), mixture of ascomycetes and mixture of ascomycetes with *S. androsaceus*. Inoculation agar discs (approximately 5 mm in diameter) of fungal mycelium were cut from the leading edge of actively growing colonies on half-strength potato dextrose agar (50% PDA, Sigma-Aldrich Co., St. Louis, USA). Twenty-four discs were placed regularly all over the surface of the needle layer in systems with individual strains. Four discs of each strain were placed on the surface of the needle layer in mixture systems. All variants were performed with six replications. Glucose content in one agar disc was estimated to be 0.3 mg. Its effect on fungal growth was relatively important. In a parallel experiment we inoculated spruce needles with a mixture of blended mycelium in sterile water. The absence of easily available nutrients was crucial for the initialization of needle colonization and most of the needles remained sterile (data not shown). During incubation, litter moisture was adjusted by adding sterilized distiled water in 14-day intervals. Air dried fresh needles and sieved O_f needles served as a control for the C:N and ^{31}P NMR measurement.

Systems were harvested 5.5 months after inoculation. Prior to chemical analysis, fungal colonization was estimated using the reisolation method. This reisolation was adopted for the following purposes: (i) to confirm that individual fungi could colonize the needles (especially ascomycetes, whose mycelium was not observed directly on the needles) and therefore changes in phosphorus refer directly to fungal activity; (ii) to assess competition among fungal strains in the mixture variant. A grid (2 cm grid square size) was projected on the surface of the litter layer. Single needles were aseptically taken from the grid's interception points from the upper and bottom surfaces of the layer (74 needles per dish). Needles were placed on malt extract agar (1.5% MEA, Sigma-Aldrich Co.) and outgrowing mycelia were identified comparing them with reference cultures. The relative area colonized by individual fungi in a particular system was estimated as a proportion of needles colonized by this strain out of all needles sampled from the system and expressed in percents. Relative areas counted separately for both the upper and bottom surface were statistically analysed (one-way ANOVA with "fungal species" as dependant factor; SPSS statistical software).

2.2. Chemical analyses, ^{31}P NMR

Prior to further analyses, needles from all replicates within one variant were pooled into one representative

sample, then air-dried and crushed to particles <0.25 mm. Weight loss of needles in systems was not measured because of the need to remove needles for reisolation. A small part of the needle sample was ground to a fine powder and analysed for total phosphorus, carbon and nitrogen content. Total phosphorus (P_t) was determined after sample digestion in 72% $HClO_4$ (Sommers and Nelson, 1972) spectrophotometrically at 660 nm as the phosphomolybdate blue (Murphy and Riley, 1962; Watanabe and Olsen, 1965). Total carbon and nitrogen were determined using Carlo Erba 1110 CHN analyzer.

Alkaline extracts were prepared using the 0.1 M NaOH solution after decalcification by 0.1 M HCl. Total extractable phosphorus (P_e) was determined in NaOH extracts using the same method as P_t. Alkaline extracts were concentrated, washed using distiled water in an Amicon ultrafiltration cell with membrane type YM1 and freeze-dried. To determine phosphorus loss during nanofiltration, the filtrate was dried, weighed and phosphorus forms were determined. In a separate part, total phosphorus was determined after mineralization with $HClO_4$. The loss was negligible, did not exceed 8.2% and consisted of two main phosphorus forms—orthophosphate and phosphate monoesters.

The ^{31}P NMR spectra of extracts diluted in 0.1 M NaOD were recorded on Bruker DRX 400 spectrometer operating at a frequency of 202.45 MHz using the following parameters: recycle delay of 2 s, number of scans 10,000; broad-band decoupling. The free-induced decay (FID time-domain signal) was processed with line broadening of 10 Hz, chemical shifts were measured relative to an external standard (85% H_3PO_4). Spectra interpretation was based on literature assignment (Novák et al., 2005a). Quantitative analysis was achieved using instrumental integration of NMR peaks. The content of individual phosphorus forms was expressed relative to the total extracted phosphorus (P_e) content. A mixture of the 0–2-year-old spruce needles, collected from living trees in the same stand, was also included to define total phosphorus, carbon and nitrogen content and phosphorus forms in living needles.

3. Results

3.1. Fungal colonization

Litter needles in the majority of systems inoculated with individual fungal strains were intensively colonized after cultivation with the exception of systems inoculated with *C. longipes* (needles remained almost intact, as fungal growth was limited only to several needles around the agar discs and were not included in further analysis). Aerial mycelia of *M. minutella* and NK11 were visible on the upper surface of the needle layer. Dense white mycelium of *S. androsaceus* colonized the entire litter layer and rhizomorphs and even minute fruitbodies were observed frequently. Intensive colonization of the other fungi was confirmed after the reisolation test, because of the mycelial growth from all needles placed on agar plates.

Needles in mixture systems were also intensively colonized and reisolation revealed significant differences in colonized area among fungal species ($p<0.05$). Both *S. sphagnicola* and *S. androsaceus* dominated and were reisolated in comparable frequencies from the upper and bottom parts of the needle layer (Table 1). The unknown ascomycete NK11 was negatively affected by the presence of *S. androsaceus* in the mixture variant and was reisolated at less frequency than from the mixture of ascomycetes. *M. minutella* and *C. pinastri* were outcompeted in the mixture variant with *S. androsaceus* and were not reisolated at all.

3.2. Needle decomposition

The C:N ratio of freshly fallen needles reached 39.8 (data from Kovářová and Vacek, 2003). The ratio decreased in control needles collected from the O_f horizon to 25.8. A further shift of C:N ratio during incubation in systems varied among variants colonized by individual fungal strains or their mixture. The greatest decrease was recorded in the variant with *S. androsaceus* (11.3) followed by the mixture variant of ascomycetes with *S. androsaceus* (21.9). Systems with single strains of ascomycetes and their mixture showed negligible change in the ratio (Table 2).

Table 1
Proportions of spruce needles colonized by selected model fungal strains after reisolation from mixture systems (mean values, $n = 6 \pm$ SEM)

	Reisolated needles (%)					
	M. minutella	*S. androsaceus*	*S. sphagnicola*	*C. pinastri*	NK11	*C. longipes*
Mixture of ascomycetes						
Upper	8.6±1.29	—	53.6±2.14	2.3±0.57	35.6±2.08	0
Bottom	2.5±0.88	—	69.8±2.60	1.6±0.65	26.1±2.58	0
Mixture of ascomycetes + *S. androsaceus*						
Upper	0	64.5±9.67	24±3.09	0	11.5±7.67	0
Bottom	0	63.5 ±11.66	16.9±3.68	0	19.6±10.76	0

0, not reisolated;—, not inoculated in the system.

3.3. Differences in phosphorus composition

Phosphorus concentration in extracts from fresh spruce needles reached 2186 mg kg^{-1} and litter needles 2326 mg kg^{-1}. Phosphorus concentration in extracts from incubated litter needles varied between 1476 and 4795 mg kg^{-1}, with the highest concentration in the needles incubated with *M. minutella* (Table 2). The solution ^{31}P NMR spectrum of fresh spruce needle NaOH extract was characterized by two main resonance peaks of phosphate-monoester at 4–4.7 ppm and phosphate-diesters (mainly DNA) near 0 ppm. A small band at 5.9 ppm corresponded to the inorganic orthophosphate; the phospholipids band near 1.6 ppm was negligible. Compared with fresh spruce needles, the spectra of needles from the control variant (partially decomposed needles from O_f horizon) contained similar phosphorus forms as the extract from fresh needles, enriched by about 13% diphosphates and 6% polyphosphates, resonating at −5 and −20 ppm, respectively. A part of organic phosphorus was mineralized to orthophosphate during litter decomposition (Fig. 1).

The loss of monoesters with simultaneous increase in diphosphates, polyphosphates and phosphonates was attributed to microbial decomposition following the needle fall. Inoculation of O_f needles with selected fungal strains in our experiment and further phosphorus transformation confirmed fungal involvement in this process. However, fungal strains did not reveal a universal pattern of phosphorus transformation. The exact amount of phosphorus fractions differed between basidiomycetes and ascomycetes and also within this group (Fig. 2).

Needles colonized with *M. minutella* contained the highest amount of polyphosphates resonating around −20 ppm and also a substantial amount of diphosphates with signal around −5 ppm. This pattern differed from other strains of ascomycetes, namely *C. pinastri*, *S. sphagnicola* and NK11, which produced only a limited amount of polyphosphates. The negative effect of fungal competition on *M. minutella* resulted in a relatively low amount of diphosphates in the mixture variant of ascomycetes (Fig. 3). The highest amount of diphosphates accumulated by individual strains was recorded from needles colonized by *S. androsaceus* (38.4%) contrasting with the lowest amount in systems with *C. pinastri* (5.6%) (Table 3).

A single phosphonate peak in the *S. androsaceus* variant appeared around 19 ppm. This peak's clear delimitation revealed that the analysis process did not affect the phosphonate content and phosphonate degradation in hydroxide did not appear. *C. pinastri* produced a comparable proportion of phosphonates. Phosphonate traces were also recorded from needles incoulated with ascomycetes *S. sphagnicola*, *M. minutella* and NK11, but not in their mixture variant.

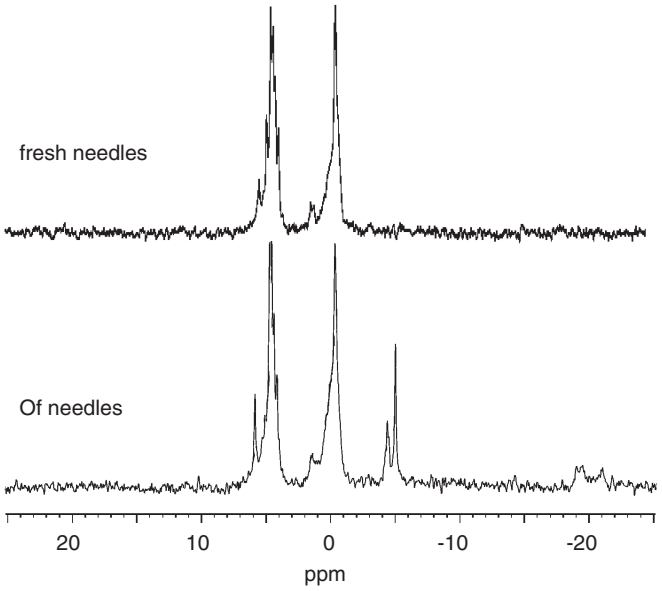

Fig. 1. ^{31}P NMR spectra of 0.1 M NaOH extracts from fresh and O_f spruce needles.

Table 2
Concentration of total phosphorus in needles (P_t) and alkaline extracts (P_e) and C:N ratio of fresh, O_f needles and needles from systems colonized with saprotrophic fungal strains and their mixtures

Needle type	P_t (mg kg^{-1})	P_e (mg kg^{-1})	C:N
Inoculated systems			
C. pinastri	981	1476	26.1
M. minutella	1331	4795	24.5
NK11	1202	2553	27.7
S. androsaceus	2479	3692	11.3
S. sphagnicola	1077	1675	27.2
Mixture of ascom.	1195	2531	25.6
Mixture of ascom. with *S. androsaceus*	1534	2855	21.9
Control needles (O_f)	1158	2186	25.8
Fresh needles	1379	2326	39.1[a]

[a]From Kovářová and Vacek (2003).

4. Discussion

4.1. Fungal competition in mixture systems

Litter needles are colonized by hundreds of fungal species and there is only limited evidence of the role of particular fungi in decomposition or nutrient transformation. Simulation of fungal decomposition with single fungal strains in laboratory conditions brought valuable data about the effect of a particular fungus (Ghosh et al., 2003). Comparably, needles pre-inoculated with selected fungal strains and then placed in the forest soil also simulated the effect of fungal competition on decomposition (Cox et al., 2001). Our study intended to combine these two approaches.

Fungal strains previously isolated from spruce litter needles and then introduced into the same substrate could colonize litter needles in systems and compete for space.

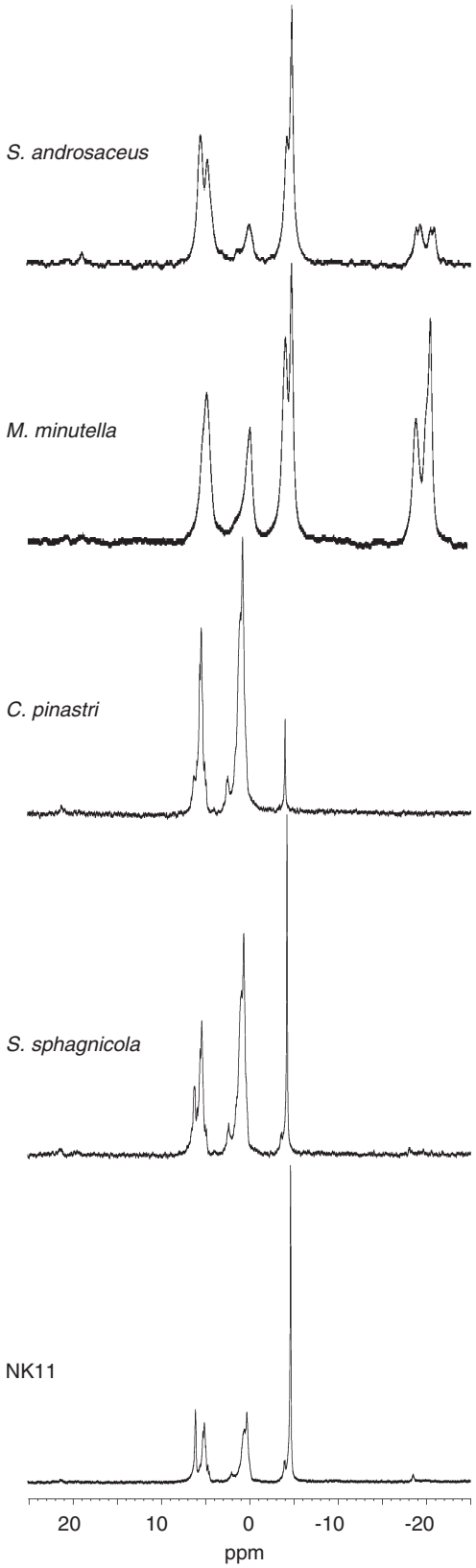

Fig. 2. ^{31}P NMR spectra of 0.1 M NaOH extracts from spruce needles colonized by selected fungal strains.

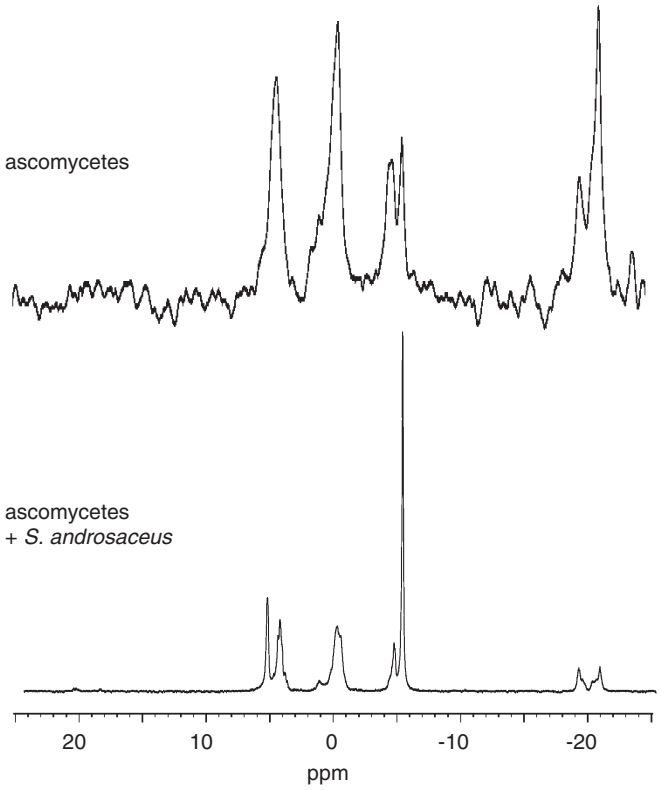

Fig. 3. ^{31}P NMR spectra of 0.1 M NaOH extracts from spruce needles colonized by mixture of fungal strains.

S. sphagnicola, which we reisolated in abundance from mixture systems with ascomycetes is supposed to be highly tolerant to biotic stress during interactions with other fungi (Hambleton et al., 2003). The strain of *S. androsaceus* introduced to the mixture of ascomycetes dominated among reisolated strains, as was expected, because *S. androsaceus* widely distributed on litter needles is a key species responsible for spruce litter decomposition (Gourbière and Corman, 1987; Ponge, 1991). It also possesses both cellulolytic and ligninolytic activity (Cox et al., 2001). The tested strain, however, could not completely replace the other strains. The reisolation test revealed viable mycelium of the strain NK11 and mycelium and conidia of *S. sphagnicola*.

Results from the reisolation support our previous competition study using agar plates with nutritionally poor media made from spruce needles, where *S. androsaceus* and *S. sphagnicola* were successful competitors (Koukol et al., in press). Simultaneously, chemical analysis results suggest the negative effect of fungal competition on decomposition of spruce needles. Lower decrease of C:N ratio in systems inoculated with ascomycetes and *S. androsaceus* compared to systems inoculated solely with *S. androsaceus* corresponds to the lower proportion of polyphosphates and phosphonates from mixture systems (Fig. 3). These data obtained from mixture variants have better relevance to natural conditions.

Table 3
Phosphorus fractions in NaOH extracts from fresh spruce needles, O_f needles and needles colonized with saprotrophic fungal strains and their mixture

Needle type	Distribution of phosphorus fractions, % of P_e						
	Phosphonates (18–20 ppm)	Inorganic ortho-phosphate (5.5–7 ppm)	Phosphate monoesters (3–5.5 ppm)	Phospholipids (1.5–3 ppm)	Phosphate diesters (1.5–(−2) ppm)	Diphosphates (−3.5–(−5) ppm)	Polyphosphates (−6–(−23) ppm)
Systems inoculated with							
C. pinastri	1.8	3.5	26.7	3.9	58.6	5.6	0
M. minutella	0.7	1.1	18.6	1.1	13.3	33.0	32.3
NK 11	0.4	12.2	18.1	2.7	29.8	34.6	2.2
S. androsaceus	1.9	17.4	16.5	3.3	7.3	38.4	15.3
S. sphagnicola	0.7	3.3	21.1	3.6	51.0	20.1	0.3
Mixture of ascom.	0	0	20.8	1.6	29.1	18.1	30.3
Mixture of ascom. with *S. androsaceus*	0.7	11.3	16.4	2.0	23.6	35.5	10.4
Control needles (O_f)	0	5.9	34.8	3.7	36.7	13	6
Fresh needles	0	0	52.1	1.1	46.8	0	0

4.2. Formation of polyphosphates and diphosphates during fungal decomposition

In natural conditions, polyphosphate accumulation is highly important for the phosphorus cycle. Polyphosphates have many important functions in organisms. Above all, they may serve as a phosphorus source for biosynthesis of nucleic acids and phospholipids. Polyphosphate accumulation in cells minimizes both increasing osmotic pressure in comparison with phosphate accumulation and interactions of inorganic phosphate and nucleotide. Moreover, they can function as an energy storage source or buffer against pH stress (see Chen, 1999). According to Harold (1960), high accumulation of polyphosphates may indicate a stationary phase of fungal growth during unfavourable growth conditions, when RNA is degraded and serves as a phosphorus source for polyphosphate synthesis. Similarly, Katchmann and Fetty (1955) found that low polyphosphate content during the exponential phase of yeast growth is followed by polyphosphate accumulation in the stationary phase. Matsunaga et al. (1982) described characteristic polyphosphate accumulation in the stationary phase of cultivation for the mitosporic fungus *Penicillium ochro-chloron*. Presented literature data are of limited relevance because fungi were cultivated on artificial media with easily utilized nutrients. E.g. Makarov et al. (2005) found high polyphosphate accumulation in most strains of soil micromycetes including three species of the genus *Penicillium* growing in liquid nutrition media. However, in our previous study (Novák et al., 2005b) we referred to a ^{31}P NMR spectrum of NaOH extract from spruce needles intensively colonized by *Penicillium* cf. *chrysogenum*, which revealed a low amount of diphospates (8.5%) and no polyphosphates. This suggests that typical members of the r-type selection and ubiquitous colonizers of various substrates (e.g. genera *Penicillium*, *Mortierella*) can accumulate polyphosphates only in laboratory conditions on artificial nutrient media. When inoculated on natural substrate, their effect on polyphosphate production is negligible, as these fungi are not considered to participate in litter decomposition.

In view of our results, we believe that the presence of polyphosphates indicates fungal decomposition, which was already suggested by Ghonsikar and Miller (1973) and widely accepted by other authors (Bedrock et al., 1994; Turner et al., 2004b). Absence of polyphosphates, however, does not confirm missing fungal activity. Needles from systems inoculated with *C. pinastri* failed to produce polyphosphates and systems with *S. sphagnicola* revealed only trace amounts. Our results do not allow us to support the theory of polyphosphate accumulation during the stationary growth phase, as we have limited evidence that fungi reached the phase. We can only hypothesize that relatively fast growing strains *S. androsaceus* and *M. minutella*, which thoroughly colonized litter needles soon before the harvesting time, reached the stationary phase and thus accumulated the highest amounts of polyphosphates (Fig. 2).

4.3. The nature and role of phosphonates

Another form of phosphorus, the phosphonates (phosphonolipids), are of particular interest, as their nature in soils remains obscure. A variety of organisms such as bacteria, amoeba, fungi and snails are most probably responsible for phosphonate production because they can all produce 2-aminoethyl phosphonic acid (Hilderbrand and Henderson, 1983). Phosphonates, however, are relatively labile to microbial degradation. Thus, their presence has been ascribed mostly to wet, cold, or acidic soils where the activity of microorganisms capable of synthesising phosphonatase enzymes and utilizing phosphonates was inhibited (Tate and Newman, 1982). Because of phosphonate lability and the extracting method, only trace quantities were recorded in pasture soils of England and Wales and the phosphonate amount was proportionally

greatest where the microbial biomass was the smallest (Turner et al., 2003). In a further study, Turner et al. (2004a) regularly found phosphonates in birch forest litter samples from Sweden and sporadically in tundra litter and soil samples. They hypothesised that the absence of phosphonates in tundra samples was caused by higher pH than in the forest samples resulting in rapid phosphonate degradation. Surprisingly, the high phosphonate content found in humic acids isolated from the A_t soil horizon of a mountain spruce forest was described in our previous study (Novák et al., 2000).

In our study, all five strains of litter colonizing fungi produced at least traces of phosphonates. Substantial phosphonate accumulation by the strain *S. androsaceus* confirmed the results of our previous study in which needles colonized by *S. androsaceus* strain MA01 contained 3.4% of total extracted phosphorus bound in phosphonates (Novák et al., 2005b).

We concluded based on our results that phosphonate presence in soil or litter samples may be ascribed to fungal activity. Most probably, dominant fungal species in a particular substrate may produce and accumulate phosphonates during favourable microclimatic conditions. Taxonomic delimitation of phosphonate-producing fungal species does not seem valid. The proportion of phosphonates in needles inoculated with *C. pinastri* was comparable to those inoculated with *S. androsaceus*. Literature data suggest the phosphonate presence also in oomycetes, e.g. Wassef and Hendrix (1977) isolated phosphonates from fungus *Pythium prolatum*. Makarov et al. (2005), however, found no phosphonates in extracts from 12 studied soil micromycetes (genera *Acremonium*, *Mortierella*, *Penicillium*, *Tolypocladium*, *Trichoderma* and *Trichurus*).

Similarly, the function of phosphonates in organisms has not been adequately explained. Perhaps, phosphonolipids as cell-membrane structural elements play an important role in membrane permeability and stabilization, or can protect them from the activity of phospholipases (Mukhamedova and Glushenkova, 2000). Our knowledge about the presence of phosphonates in plants is insufficient probably due to the low content in plant tissue. We found no phosphonolipids in the extract from fresh spruce needles, which is in agreement with Makarov et al. (2002, 2005).

5. Conclusions

Selected strains of saprotrophic fungi colonizing litter needles transformed phosphorus forms present in spruce litter needles from orthophosphate monoesters and diesters into diphosphates, polyphosphates and phosphonates. These results confirmed that both basidiomycetes and ascomycetes are involved in phosphorus transformation. It is highly probable that the dominant species *S. androsaceus* contributes considerably not only to the needle decomposition but to the phosphorus cycle in coniferous litter as well.

The presence of phosphonates in needles colonized by fungal strains implies a more extensive study of P_{org} composition in spruce litter decomposed by various fungi. This should verify if phosphonate synthesis and accumulation are characteristic for a certain taxonomical or ecological group of fungi. Other facts may affect the phosphonate signal, as they are easily degradable in strong alkali. Thus, (i) the use of concentrated NaOH should be avoided during extract preparation and (ii) the process of ^{31}P NMR spectra measurement should be relatively short.

Acknowledgements

This work was supported by the Grant Agency of the Czech Republic and by Research Plan "Relationships between the structure and function of decomposer food web in soil" of the Institute of Soil Biology, ASCR.

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