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Soil organic matter transformations induced by *Hieracium pilosella* L. in tussock grassland of New Zealand

Abstract To study the effect of *Hieracium pilosella* L. invasion on the transformations of soil organic matter of New Zealand tussock grassland soils (Ustochrepts), plant material and soils underneath *Hieracium*, the surrounding halo, and the adjacent herbfield (depleted tussock grassland) were examined for their chemical composition. An attempt was made to reveal possible changes in chemical composition of the soil organic matter induced by *H. pilosella* invasion. Small differences were detected by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy in the composition of the plant and soil materials from these zones. Most of the differences in soil organic matter occurred due to differences in the amount and quality of plant-residue inputs. Comparable amounts of phenolic C were detected in the solid-state ^{13}C NMR spectra of *H. pilosella* and herbfield vegetation, while alkaline CuO oxidation yielded considerable lower lignin oxidation products for *H. pilosella*. A slightly higher proportion of these compounds in *H. pilosella* soil revealed an accumulation and a low degradation rate of lignin compounds under *H. pilosella*. The HCl hydrolysis and solid-state ^{15}N NMR spectroscopy showed similar chemical compositions of the N fractions of the three different soils. The absence of ^{15}N NMR signal intensity assignable to aniline derivatives or aromatic heterocyclic N indicates that the condensation of phenolic compounds with N groups plays a minor role in N sequestration in these soils.

Key words Carbon-13 nuclear magnetic resonance · Nitrogen-15 nuclear magnetic resonance · Lignin · Phenols · Organic nitrogen

Introduction

The background of this study is described by Saggar et al. (1999) who compared soil microbial biomass C, N and P contents, metabolic quotient and organic matter turnover beneath *Hieracium pilosella* L. and its adjacent herbfield (depleted tussock grassland). Soil processes under *H. pilosella* were found to be profoundly different from those occurring in herbfield soils because of the differences between soil microbial biomass and organic matter pools and dynamics under the two vegetation types. Laboratory incubation studies showed an enhanced organic C turnover and a considerable reduction of net N mineralization in the soils under the *H. pilosella* patches relative to those of the halo and the surrounding herbfield. A reduction of N mineralization was proposed to be related to polyphenols and lignins released by *H. pilosella* (Saggar et al. 1999).

In this paper we evaluate the nature and structure of organic matter of these soils and their associated plants (herbage and roots) using solid-state ^{13}C and ^{15}N nuclear magnetic resonance (NMR) spectroscopy, lignin composition and hydrolyzable and non-hydrolyzable N contents to further elucidate the possibility of polyphenol-protein complex formation.

Materials and methods

Soil and plant sampling and analysis

Soil and plant samples were obtained from the Glencairn Station near Twizel, South Island, New Zealand (McIntosh and Allen 1993). A full description of the site, soils and soil sampling strategy used in this study is presented elsewhere (McIntosh et al. 1995; Saggar et al. 1999). Briefly, the site at 440 m above sea level re-

ceives 500–600 mm annual rainfall. Soils are developed on thin loess over bouldery fan alluvium derived from greywacke and classified as Immature Pallic soils (Hewitt 1992) and Typic Ustochrepts (USDA 1998). The *Hieracium* typically occurs as patches in the herbfield (depleted tussock grassland), with a non-vegetated halo around each patch. Soil samples (0–10 cm depth) were collected from the patch centre, the halo and the adjacent herbfield at four sites. Herbage and roots of the growing vegetation were included at the time of sampling. The herbage was removed and the root material was handsorted from the soil. Soil samples were sieved (<2 mm) and air dried for further analysis.

Total C and N content of soils, herbage and roots were determined with an Elementar Vario EL elemental analyzer in duplicates with a mean coefficient of variation of 5.4% for N and 7.2% for C. Soil pH was determined potentiometrically in 0.01 M $CaCl_2$ at a soil:solution ratio of 1:2.5. Cation-exchange capacity (CEC) and exchangeable cations were measured with an unbuffered 0.5 M NH_4Cl solution according to Trüby and Aldinger (1989).

NMR spectroscopy

Prior to NMR spectroscopy, the soil samples were treated with 10% hydrofluoric acid (HF) solution in order to decrease the content of paramagnetic materials and to enrich the organic matter content by removal of mineral matter (Schmidt et al. 1997). Five grams of each sample were weighed into 50 ml polyethylene beakers. After the addition of 40 ml of 10% (v/v) HF the closed beakers were shaken overnight and subsequently centrifuged. The supernatant was carefully removed with a tube attached to a plastic syringe to prevent the loss of fine material by decanting. The HF treatment was repeated 3 times. The residues were washed with distilled water to remove HF and vacuum filtered on 0.45 μm (pore size) filters. The isolated material was freeze-dried.

The solid-state ^{13}C NMR spectra were obtained on a Bruker DSX 200 operating at a frequency of 50.3 MHz using zirconium rotors of 7 mm outer diameter with KEL-F caps. The cross-polarization magic-angle spinning (CPMAS) pulse sequence (Schaefer and Stejskal 1976) was applied. The rotor was spun at 6.8 kHz. A contact time of 1 ms and a 90° ^1H pulse width of 4.3 μs were used. The ^{13}C-chemical shifts were calibrated to tetramethylsilane (=0 ppm). Between 10,000 and 30,000 scans were accumulated using a pulse delay of 400 ms and 4 s for the bulk soils and the plant material, respectively (Fründ et al. 1989; Knicker and Lüdemann 1995). A line broadening between 20 to 75 Hz was applied. Relative C distribution was determined by the integration of signal intensity in the various chemical shift regions with an integration routine supplied with the instrument software.

The solid-state CPMAS ^{15}N NMR spectra were obtained on a Bruker DMX 400 operating at 40.56 MHz with the application of a contact time of 1 ms, a 90° ^1H pulse width of 5 μs, a pulse delay of 150 ms (soil) and of 300 ms (plant material), and line broadening between 100 Hz and 200 Hz. Between 1.5 million and 3 million scans were accumulated at a magic-angle spinning speed of 5.5 kHz. For all spectra, zirconium rotors of 7 mm outer diameter with KELF-F caps were used. The chemical shift was referenced to the nitromethane scale (=0 ppm) and was adjusted with ^{15}N-labelled glycine (–347.6 ppm).

Lignin analysis

Lignin was analysed by the CuO oxidation method (Kögel and Bochter 1985). Samples (50 mg plant material, 500 mg soil) were oxidized with 250 mg CuO and 2 M NaOH at 172 °C under N_2 for 2 h. After oxidation, a standard containing ethylvanillin was added to elucidate the recovery of lignin products after the whole procedure (72–83%). The solution was quantitatively transferred to glass beakers, adjusted to pH 1.8–2.2, and the generated lignin-derived phenols were cleaned by a C18 column. The eluate was dried under N_2 and silylated for gas chromatography (Hedges and Ertel 1982). The lignin oxidation products were separated and detected with a Fisons GC 8000 gas chromatograph, equipped with a J and W DB-5 column (15 m length, 0.25 mm inner diameter, 0.25-μm coating) and flame ionization detector. The analysis was carried out in duplicate. A mean coefficient of variation of 14.6% was found for the determination of individual phenols.

N fractions

For the determination of the amount of α-amino acids, samples were hydrolysed with 6 M HCl for 12 h at 105 °C in duplicate (Kögel-Knabner 1995). The hydrolyzates were filtered and washed with distilled water. The filtrates were freeze dried. The supernatants were boiled for 20 min in a water bath after the addition of a sodium citrate solution and ninhydrin reagent. The concentration of purple complexes formed by the reaction of ninhydrin with α-amino acids was determined photometrically at 570 nm. The N content of the hydrolysis residues constituted non-hydrolyzable N. The hydrolyzable but not identified N fraction was calculated as the difference between total N and the sum of amino N and non-hydrolyzable N. The mean coefficient of variation was 9.6%.

Carbohydrate analysis

Duplicate samples were hydrolysed with 72% H_2SO_4 at room temperature for 16 h. The concentration of the H_2SO_4 solution was diluted to 0.5 M with distilled water and heated to 100 °C for 5 h. The amount of extracted monosaccharides was photometrically determined after reaction with 3-Methyl-2-benzo-thiazolinon-hydrazon-hydrochloride-hydrate (MTBH) according to the procedure described by Johnson and Sieburth (1977) and Kögel-Knabner (1995). The yields of non-cellulosic carbohydrates were measured photometrically after hydrolysis with 1 M HCl at 100 °C for 5 h and reaction with MTBH. The content of cellulose was calculated from the difference between total carbohydrates and non-cellulosic carbohydrates. The mean coefficient of variation was 3.9% for non-cellulosic carbohydrates and 3% for total carbohydrates.

Results and discussion

Soil properties

As reported previously (McIntosh et al. 1995; Boswell and Espie 1998; Saggar et al. 1999), soil organic C and N contents were higher under *Hieracium* than under the halo or herbfield. This difference was more pronounced for organic C than total N, resulting in considerable changes in the C:N ratio (Table 1). The pH values were different among the soils, showing a distance gradient from the centre of the *H. pilosella* patches (4.7) to the herbfield (5.3) (Table 2). This indicates the ability of *H. pilosella* to acidify soils compared to the adjacent grassland vegetation and is in accordance with previous studies (McIntosh and Allen 1993; McIntosh et al. 1995; Boswell and Espie 1998). The CEC followed an opposite trend to soil pH. This was probably the result of the high amount of protons (13.3 mmol kg^{-1}) at the exchange sites of the soils under *H. pilosella*, because no protons were detected in the halo and herbfield soils. Furthermore, *Hieracium* soils had lower base saturation (80%) compared with the halo and herbfield soils (97%). All the exchangeable cations showed mi-

Table 1 C and N content of the herbage, roots and soils underneath *Hieracium pilosella*, halo around *H. pilosella* and adjacent herbfield; means ± SD ($n = 4$). *HF* Hydrofluoric acid

	C (%)	N (%)	C:N ratio
Herbage			
Hieracium	31.7 ± 3.8	0.92 ± 0.07	33.9 ± 4.4
Adjacent herbfield	28.6 ± 4.2	0.78 ± 0.07	37.0 ± 6.9
Roots			
Underneath *Hieracium*	36.8 ± 1.1	0.78 ± 0.26	50.4 ± 13.8
Halo around *Hieracium*	31.7 ± 1.7	0.68 ± 0.07	47.0 ± 5.6
Adjacent herbfield	25.3 ± 1.5	0.72 ± 0.07	35.5 ± 2.6
Soil (before HF treatment)			
Underneath *Hieracium*	2.05 ± 0.18	0.15 ± 0.01	13.3 ± 0.4
Halo around *Hieracium*	1.50 ± 0.10	0.12 ± 0.01	12.3 ± 0.2
Adjacent herbfield	1.63 ± 0.13	0.14 ± 0.01	11.7 ± 0.2
Soil (after HF treatment)			
Underneath *Hieracium*	12.9 ± 1.8	1.04 ± 0.08	12.5 ± 0.8
Halo around *Hieracium*	10.0 ± 0.8	0.91 ± 0.07	11.0 ± 0.4
Adjacent herbfield	10.6 ± 0.7	0.99 ± 0.05	10.7 ± 0.3

nor differences among three soils except K and Mn. K increased with distance from the *H. pilosella* patches to the herbfield (from 4.4 to 6.9 mmol kg^{-1}) which may be partly due to a higher uptake of K by *H. pilosella* compared to the herbfield (Davis 1997; Boswell and Espie 1998), and partly to leaching effects due to proton competition from the soil matrix under the *Hieracium*. Higher exchangeable Mn under *Hieracium* appeared to be the result of increased acidity.

C fractions of plant materials

The solid-state CPMAS ^{13}C NMR spectra of the plant and the root material of *Hieracium* and the herbfield are given in Fig. 1. The highest signal intensity (68%) for the herbfield spectra detected in the 110–60 ppm region is assigned to O-alkyl-C (Table 3). The spectra of *Hieracium* plants and the root material have a slightly smaller intensity (64% and 58%, respectively) in this region. The dominance of the peak at 72 ppm is typical for C2, C3, C4 of carbohydrates and their corresponding anomeric C results in a signal at 104 ppm. The signal of their C6 observed around 64 ppm was best resolved in the spectrum of the herbfield vegetation. The signal between 160 ppm and 140 ppm is assigned to phenol-C, as phenolic compounds occur in lignin (C3/C4 in guaiacyl; C3, C4, C5 in syringyl; C4 in *p*-hydroxyphenyl) (Lüdemann and Nimz 1974) or in tannins (Preston et al. 1997). The latter, however, would also contribute to the signal at 104 ppm. Sp^2 hybridized C of C-substituted or non-substituted aromates or olefines contribute to the region between 140 ppm and 110 ppm. No large differences in relative intensity were apparent for the region of aromatic C (160–110 ppm) in the spectra of *H. pilosella* and herbfield vegetation (Fig. 1 and Table 3). Carboxyl-C of esters, amides and free carboxylic groups represent the signal between 185 ppm and 160 ppm. The corresponding C in the positions of amino acids are expected to result in resonance lines between 60 ppm and 45 ppm, which is overlapped by the dominating signal at 72 ppm. The methoxyl groups of the lignin or hemicelluloses units can also contribute to this region. The absence of a sharp peak at 56 ppm in the spectra (Fig. 1) indicates the minor contribution of methoxyl groups to this region. The chain C of amino acids, paraffins or fatty acids appear between 45 ppm and 0 ppm. The small signal between 220 ppm and 185 ppm lies in the chemical shift region of aldehydes and ketones, but is most probably assigned to a spinning side band of the main peak at 72 ppm.

With the MBTH method, approximately 45% of the total C of the herbfield plant material and 26% of that of *H. pilosella* was identified as carbohydrate C (Table 4), approximately one-third to one-quarter of which can be assigned to cellulose. The amounts of carbohydrates that were colorimetrically determined explain approximately two-thirds of the relative intensity observed in the chemical shift region of O-alkyl-C of the corresponding solid-state ^{13}C NMR spectra of the herbfield plant material, and approximately one third of those of *H. pilosella*. The higher amount of O-alkyl-C determined with NMR spectroscopy relative to the carbohydrate yields obtained by the photometrical approach may be explained by the fact that signals of ether and OH-substituted C of compounds other than carbohydrates can contribute to the intensity of the chemical shift region between 110 ppm and 60 ppm. Alterna-

Table 2 Mean pH, nutrient content and cation-exchange capacity (*CEC*) of the soils underneath *H. pilosella*, halo around *H. pilosella* and adjacent herbfield; means ± SD ($n = 4$). *n.d.* Not detectable

Sample	pH (1:2.5)	Mn	Mg	Na	K	Ca	H	CEC	Ca + K + Mg + Na	Base saturation (%)
		(mmol kg^{-1} dry soil)								
Underneath *Hieracium*	4.7 ± 0.05	4.4 ± 0.93	18.3 ± 2.68	0.2 ± 0.03	4.4 ± 0.84	49.8 ± 4.84	13.2 ± 3.92	90.5 ± 5.51	72.8 ± 8.01	80 ± 4.48
Halo around *Hieracium*	5.1 ± 0.13	2.3 ± 0.93	17.6 ± 1.30	0.3 ± 0.09	5.1 ± 0.82	47.2 ± 4.51	n.d.	72.5 ± 5.01	70.2 ± 4.25	97 ± 1.08
Adjacent herbfield	5.3 ± 0.16	2.5 ± 1.33	20.4 ± 3.37	0.3 ± 0.13	6.9 ± 2.38	50.2 ± 4.59	n.d.	80.3 ± 6.48	77.8 ± 5.96	97 ± 1.59

Table 3 Mean relative intensity distribution (%) in the solid-state ^{13}C NMR spectra of the herbage, roots and the soils of *H. pilosella*, their surrounding halos and tussock grassland obtained from the Glencairn Station near Twizel, South Island, New Zealand; means ± SD ($n = 4$). *ssb*$^+$ Spinning side bands of the signal at 72 ppm

Sample	220–185 ppm (Carbonyl-C, ssb$^+$)	185–160 ppm (Carboxyl/amide/ester-C)	160–140 ppm (Phenol-C)	140–110 ppm (sp^2–Hybridized-C)	110–60 ppm (O-Alkyl-C)	60–45 ppm (CH_3O-C/N-alkyl-C)	45–0 ppm (Alkyl-C)
Herbage							
Hieracium	1.4 ± 0.6	5.4 ± 1.8	2.7 ± 0.6	6.9 ± 0.7	58.0 ± 4.1	7.1 ± 1.0	18.5 ± 1.4
Adjacent Herbfield	1.4 ± 0.2	2.9 ± 1.0	2.5 ± 0.4	6.3 ± 0.1	68.6 ± 2.2	8.2 ± 0.3	10.0 ± 1.0
Roots							
Hieracium	0.5 ± 0.2	3.7 ± 0.2	2.6 ± 0.0	7.7 ± 1.0	64.2 ± 2.0	9.3 ± 0.3	13.1 ± 1.3
Adjacent herbfield	0.8 ± 0.1	2.3 ± 0.3	2.8 ± 0.4	7.9 ± 0.3	68.3 ± 0.6	9.0 ± 0.8	8.9 ± 0.7
Soil							
Underneath *Hieracium*	1.2 ± 0.4	7.8 ± 0.4	3.7 ± 0.1	11.7 ± 0.3	38.0 ± 1.1	10.4 ± 0.3	27.1 ± 0.7
Halo around *Hieracium*	1.7 ± 0.8	8.9 ± 0.6	4.1 ± 0.4	13.2 ± 0.7	34.2 ± 1.1	10.3 ± 0.9	27.6 ± 1.7
Adjacent herbfield	0.7 ± 0.2	7.6 ± 0.3	4.0 ± 0.3	12.9 ± 0.2	39.2 ± 0.8	10.7 ± 0.2	24.9 ± 0.5

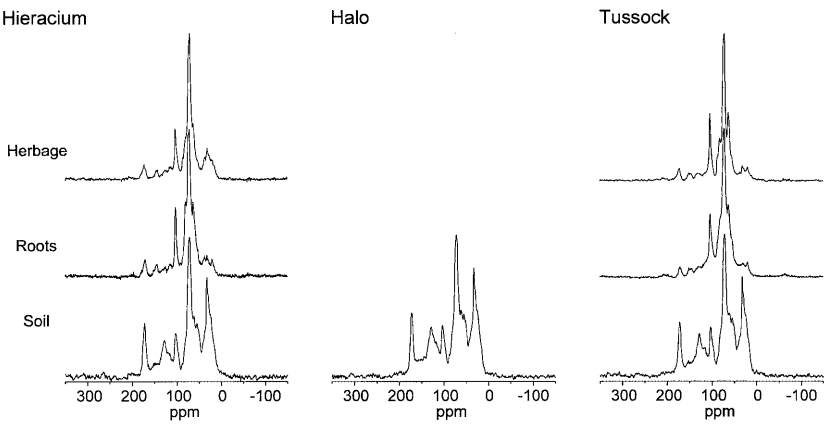

Fig. 1 Solid-state ^{13}C NMR spectra of the herbage, roots and soils underneath *Hieracium pilosella*, halo around *H. pilosella* and adjacent herbfield

Table 4 Carbohydrate content of the herbage, roots and soils underneath *H. pilosella*, halo around *H. pilosella* and adjacent herbfield; means ± SD ($n = 4$)

Sample	Non-cellulose	Cellulose	Total carbohydrates
	(mg carbohydrate-C g^{-1} organic C)		
Herbage			
Hieracium	189.7	96.7	286.4
Adjacent herbfield	333.3	120.6	453.9
Roots			
Hieracium	205.7	59.8	265.5
Adjacent herbfield	316.2	140.6	456.8
Soil			
Underneath *Hieracium*	167.8 ± 5.7	3.5 ± 6.4	174.0 ± 3.1
Halo around *Hieracium*	151.8 ± 9.3	8.9 ± 3.1	160.8 ± 12.4
Adjacent herbfield	146.9 ± 24.7	4.0 ± 3.8	169.2 ± 5.0

tively, these results may indicate the presence of carbohydrates that were not detected by the MBTH method, either because of their resistance to hydrolysis or because they were destroyed during hydrolysis.

Approximately 10% and 3% of the total signal intensity of the solid-state ^{13}C NMR spectra are found in the region of C-substituted or unsubstituted aromatic-C and of phenol-C, respectively. A preferential occurrence of the latter was not apparent from the data for any of the samples. A higher proportion of carboxyl-C and alkyl-C was detected in the spectra of *H. pilosella* plant material compared to those of the herbfield. The ratio of alkyl-C to carboxyl-C, however, ranges between 3 and 3.6 for all samples, indicating that a major proportion of the alkyl-C occurs as short-chain acids, i.e. amino acids of peptides and proteins.

C fractions of the soils

To increase the sensitivity of the NMR experiment, the soil samples were subjected to HF treatment. As revealed from the low variation of their C:N (w/w) ratios (between 10 and 12), before and after HF treatment, it can be concluded that no major alteration of the chemical composition of the samples was induced by HF (Table 2). The relative C and N contents of 2% and 0.2% were enhanced to approximately 10–12% and 1%, respectively.

Relative to the solid-state ^{13}C NMR spectra of the plant material, those of the HF-treated samples in

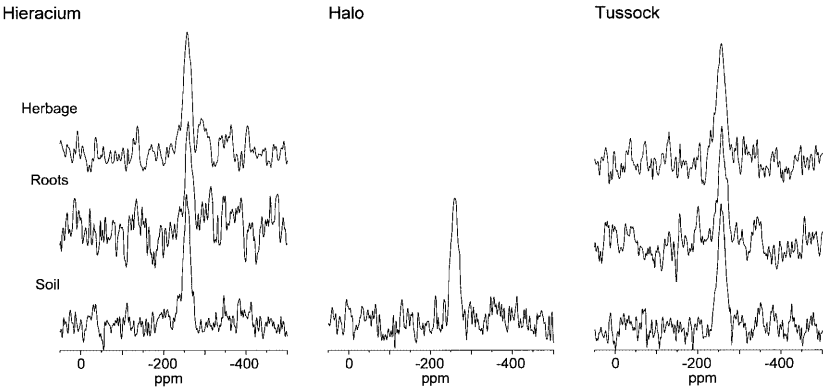

Fig. 2 Solid-state ^{15}N NMR spectra of the herbage, roots and soils underneath *H. pilosella*, halo around *H. pilosella* and adjacent herbfield

Fig. 2 show a considerable relative intensity decrease to 35–40% of the total signal intensity in the chemical shift region between 110 ppm and 60 ppm (Table 3). This relative decrease, calculated as a percentage of the mean signal intensity in the O-alkyl-C region of the spectra of the herbage and the roots, was 45%, 43% and 38% for the soil of the halo, the surrounding herbfield and beneath *H. pilosella*, respectively. This decrease in signal intensity is in accordance with the preferential degradation of carbohydrates during the early stages of humification of plant residues, leading to an accumulation of alkyl and aryl material. Applying the MBTH method, carbohydrate-C contents of 15% and 17% of total soil C were measured (Table 4), accounting for approximately one-third of the signal intensity in the O-alkyl-C region of the corresponding solid-state ^{13}C NMR spectra. Cellulosic-C detected by the MBTH methods in the soils accounted for $<4\%$ of the total C. Total carbohydrate-C decreased by 37%, 64% and 63%, respectively, for the soils under *H. pilosella,* the halo and adjacent herbfield compared with their plant materials. The lower decrease in the *Hieracium* soil compared with the halo and the herbfield soils may be the result of a higher input of fresh plant residues into the *Hieracium* soil. The higher decrease for the soil of the halo and herbfield may be the result of little or low plant-residue inputs, leaving existing organic material as a major energy source for microbial activity.

The relative increase in signal intensity in the region of aryl-C from approximately 8 to 10% in the spectrum of the plant material is most pronounced in the spectrum of the soil under the halo (17%), followed by that under *H. pilosella* (15%) and the adjacent herbfield (14%). This is in accordance with the relative accumulation of aryl-C during humification observed in other studies (Inbar et al. 1989; Kögel-Knabner et al. 1991). It is interesting to note that this increase is caused mainly by an increase in signal intensity in the 140 ppm to 110 ppm region, rather than in the typical phenol-C region. Considering the low relative intensity of $<4\%$ in the phenol-C region for all three soil spectra, it seems unlikely that phenols persist longer under *Hieracium* than under the halo or herbfield.

The amounts of carboxyl-C and alkyl-C are higher in the halo and *Hieracium* soils than in the herbfield soil. The lower value in the herbfield soils is in accordance with the lower proportion of alkyl-C in the parent plant material. The ratio of alkyl-C to carboxyl-C in all three soils is around 3, indicating a relatively short mean chain length of fatty acids. It seems that no major accumulation of long chain aliphatic material occurred during humification.

The content of phenolic lignin oxidation products after alkaline CuO oxidation amounted to 45–48.5 mg g^{-1} C [vanillin (V), syringyl (S) and coumaryl (C) derivatives] for the plant material of the herbfield (Table 5). Considerably lower yields of 11 mg and 18 mg lignin g^{-1} C were obtained for *H. pilosella* herbage and roots. Considering that the relative amount of aryl-C detected by NMR spectroscopy did not vary considerably for the plant material of *H. pilosella* and of the herbfield, these differences in lignin oxidation products may indicate that *Hieracium* contains phenolic compounds that are different from those of herbfield vegetation, and that these compounds are not detected by the CuO oxidation procedure.

In herbfield soils, the amount of lignin oxidation products decreased to <10 mg g^{-1} C, indicating that a major fraction of lignin was degraded during decomposition of the plant materials. In spite of the low amount of lignin oxidation products in the *H. pilosella* plant material, those of the underlying soil showed an accumulation (13 mg g^{-1} C), revealing low degradation rates of lignin compounds detectable after CuO oxidation in this environment. For the estimation of the lignin degradation, the relative loss of V, S and C units during humification was calculated from their average yields in the herbage and the root material and their corresponding soil organic matter. The halo and herbfield soils lost 70–85% of V, S and C units, while *Hieracium* soils lost considerably lower proportions (13–18%). Lignin degradation in soils is often accompanied by an increase in the acid:aldehyde ratios of V units and S units (Ertel and Hedges 1984; Kögel 1986). The moderate increase in these ratios from 0.11 to 0.19 in the plant material to values between 0.34 and 0.46 in the soil in-

Table 5 Lignin oxidation products, their acid : aldehyde ratios for vanillyl [$(Ac/Al)_v$] and syringyl units [$(Ac/Al)_s$] and the ratio of syringyl (*S*): vanillyl (*V*): cinnamyl (*C*) of the plant material and the soils underneath *H. pilosella*, halo around *H. pilosella* and adjacent herbfield

Sample	Syringyl (S)	Vanillyl (V)	Cinnamyl (C)	SVC	S:V:C	$(Ac/Al)_v$	$(Ac/Al)_s$
	(mg g^{-1} C)						
Herbage							
Hieracium	5.7	4.1	0.9	10.7	1.4:1:0.2	0.29	0.20
Adjacent herbfield	17.6	16.2	14.6	48.5	1.1:1:0.9	0.13	0.29
Roots							
Underneath *Hieracium*	7.1	4.7	6.4	18.2	1.5:1:1.4	0.16	0.17
Adjacent herbfield	16.9	10.5	17.3	44.7	1.6:1:1.6	0.12	0.12
Soil							
Underneath *Hieracium*	5.3 ± 0.99	4.7 ± 1.34	3.2 ± 0.79	13.0 ± 3.26	1.1:1:0.7	0.43 ± 0.07	0.34 ± 0.03
Halo around *Hieracium*	3.6 ± 0.53	4.6 ± 0.77	2.0 ± 0.47	10.2 ± 1.78	0.8:1:0.4	0.46 ± 0.11	0.46 ± 0.08
Adjacent herbfield	3.5 ± 0.19	4.0 ± 0.48	2.4 ± 0.25	9.8 ± 0.42	0.9:1:0.6	0.44 ± 0.06	0.42 ± 0.03

dicates that the lignin remaining in the soil occurs in a moderately altered oxidation state. Changes in the S:V:C ratios of the soils in comparison to the corresponding plant material show alterations in the relative distribution of these monomers during soil organic matter formation, expressed as a relative enrichment of V units (Table 5).

N fractions

Half of the total N in *H. pilosella* and herbfield shoots and roots was identified as α-amino-N, leaving approximately 10% N in the extraction residue. Approximately 40% of the total N was hydrolyzable but remained unidentified (Table 6). This N may either be derived from amino acid amides (Anderson et al. 1989) or originate from the partial destruction of amino sugars, and some amino acids of glutamine or asparagine**.** The transformation of nucleic acids during acid hydrolysis may have released additional N as NH_4^+. In the soils, only one-third of the total N was identified as α-amino-N. The lower amount of amino-N relative to the plant material is best explained by the mineralization of proteinaceous compounds during the formation of soil organic matter. The hydrolyzable but unidentified N was higher in the soil (approximately 50%) than in the plant materials (approximately 40%). This N may be partly related to indigenous soil NH_4^+, N compounds that were destroyed during acid hydrolysis, or to aniline derivatives formed during humification from the addition of amino groups to phenols. Unstable against acid hydrolysis, they may have released their N as NH_4^+. Because of the large proportions of hydrolyzable but unidentified N and variable NH_4^+ (Saggar et al. 1999) in these soils, it was not possible to differentiate aniline formation in soils beneath *Hieracium*.

Although small, the increase in the relative amount of non-hydrolyzable N in the soils compared with the plant residues may reflect an accumulation of N during soil organic matter formation. This N is often considered to represent a fraction with a high stability against further microbial degradation. It was hypothesized that this N is bound in heterocyclic aromatic structures formed after the condensation of phenolic degradation products reacted with NH_4^+ or amino groups of amino acids and sugars (Flaig et al. 1975; Kelly and Stevenson 1996). The small differences in the proportions of non-

Table 6 N fractions in the herbage, roots and soils underneath *H. pilosella*, halo around *H. pilosella* and adjacent herbfield. N_t Total N

Sample	α-Amino-N (% N_t)	Non-identified but hydrolyzable N (% N_t)	N of residue (% N_t)	C/N of residue
Herbage				
Hieracium	44	45	11	231
Adjacent herbfield	52	31	17	124
Roots				
Hieracium	55	34	11	167
Adjacent herbfield	52	31	17	144
Soil				
Underneath *Hieracium*	37	48	15	36
Halo around *Hieracium*	33	52	15	27
Adjacent herbfield	36	48	16	29

hydrolyzable N fractions in these soils indicate that such condensation reactions may not be responsible for a preferential accumulation of N in *H. pilosella* soils.

The solid-state ^{15}N NMR spectra of the plant material of *H. pilosella* and herbfield vegetation (Fig. 2), dominated by the peak around –260 ppm, suggest that peptides are the major components of organic N in plant residues. A small signal, typical for free amino groups in amino acids and amino sugars, is observed in the spectrum of herbfield roots. The absence of this signal in the other spectra is probably due to the low signal:noise ratio, which makes it difficult to distinguish signals of N functional groups with concentrations $<10\%$ from noise. In the ^{15}N NMR spectra of the HF-treated soils the dominance of the amide signal remains, confirming that most of the organic N in plant and soil is bound in peptide-like structures, most probably of biogenic origin. The lack of signals in the chemical shift region of aniline derivatives (–285 to –340 ppm) demonstrate that such compounds, if present, comprise only a minor proportion of the organic N in these soils. Heterocyclic aromatic-N, having been considered as a possible contributor to the N in the residues after acid hydrolysis, would show signals in the chemical shift region from –25 to –100 ppm (pyridine-N or imine structures) and from –145 to –250 ppm (pyrrole-N or indole-N). However, the regions between –145 and –250 ppm contain visually comparable signal intensity distribution. Thus, a preferential accumulation of indole-N or pyrrole-N in any of the soils is not revealed from these spectra. In the region of pyridine-N or imines, no signal is distinguishable from the noise, confirming the aforementioned conclusion that such compounds comprise only a small, if any, fraction of the organic N in these soils.

The ^{15}N NMR spectroscopy and HCl hydrolysis results show a similar chemical composition of the organic N fraction of the three soils. Consequently, the invasion of the herbfield by *H. pilosella* may not have a major impact on the chemical nature of the organic N fraction. The low signal intensity in the region of indole-N and pyrrole-N and the lack of considerable signal intensity assignable to aniline and pyridine analogues suggest that covalent binding between amino groups and phenolic compounds has no major role in sequestering N in these soils. It seems unlikely that this covalent binding in the soils underneath *H. pilosella* will reduce N mineralization relative to that detected for soils underneath the halo and herbfield, as obtained by Saggar et al. (1999). However, sequestration of N by phenolic compounds in soils may not be restricted to covalent binding. Proteins may also be complexed to the phenolic compounds released by *H. pilosella* by hydrophobic-hydrophilic interactions or by means of H-bridges and/or van der Waals' forces, as was suggested for tannins (Zucker 1983; Northup et al. 1995). Underneath *H. pilosella,* these interactions favoured by low pH may lead to a decrease in the accessibility of their N for microbial decay. The bonds of these interactions have no detectable impact on the chemical shift of the corresponding N in a solid-state ^{15}N NMR spectrum due to the low resolution. Thus their signals cannot be distinguished from those of the free N form. Due to the weakness of such bonds relative to covalent bonds, adsorbed N will be released during hydrolysis, contributing to the hydrolyzable fraction in the same way as expected from the free counterpart. Therefore, HCl hydrolysis will also fail to distinguish this specific N fraction from others.

In conclusion, the C fractions in these soils showed small variations in soil organic matter composition resulting from differing amounts and quality of plant-residue inputs. The determination of lignin derivatives after alkaline CuO oxidation revealed less extractable oxidation products in the *H. pilosella* herbage and the roots, although the amount of phenol-C detectable by NMR spectroscopy was comparable to that observed for the plant material of the adjacent herbfield. From these results it may be concluded that *H. pilosella* contains phenolic compounds other than lignin. Since phenolic compounds are hypothesized to be involved in soil N sequestration (Kuiters 1990; Kelly and Stevenson 1996; Saggar et al. 1999), their release during the decay of *Hieracium* litter may be involved in N accumulation.

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