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Nitrogen transformation in soil treated with ^{15}N labelled dried or composted ryegrass

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Abstract

A 5-month laboratory incubation experiment was conducted to study the immobilization-mineralization of N in soil to which dried or composted ^{15}N labelled ryegrass (*Lolium italicum* L.) had been added. Cellulose was added to dried ryegrass to give a C/N ratio similar to that of composted ryegrass. Exchangeable NH_4^+ and NO_3^-, HCl-hydrolyzable N forms, microbial biomass N, NaOH-soluble and insoluble N were monitored during incubation. Dried ryegrass brought about a significant increase in total and labelled exchangeable NH_4^+, while a rapid immobilization and a subsequent slow release of exchangeable NH_4^+ was observed in soil with composted ryegrass, together with a resistance to degradation of the labelled humic substances. Compounds synthesized during the composting process and resistant to microbial decomposition probably caused an increase in the amino-acid fraction of soil. These findings suggest that composting can reduce the risk of N losses.

Introduction

Transformations of organic N compounds in soil depend on their C/N ratios and their recalcitrance to microbial degradation; the rate of mineralization versus immobilization largely determines the amount of inorganic N, that is the form of N available to the plants and to the losses by leaching and denitrification (Azam et al., 1985a; Janson and Persson, 1982; Nugroho and Kuwatsuka, 1990; Rubins and Bear, 1942; Wagger et al., 1985).

Nitrogen immobilization occurs shortly after the application of fertilizer N to soil (Nannipieri et al., 1990) and depends on soil properties. From 1 to 10% of the applied N is immobilized in various soils after 12 hours from the application (Okereke and Meints, 1985). By adding glucose as C source together with inorganic N, the percentage of immobilized N increases to 25% after 12 hours and reaches 96% after 96 hours.

Plant-available nitrogen in organic residues and green manures added to soils has been extensively investigated (Fredrickson et al., 1982; Ladd and Amato, 1986; Müller and Sundman, 1988; Seligman et al., 1986; Sistani et al., 1989) as well as the effect of organic materials on the plant uptake of fertilizer N (Azam et al., 1985b; Kelley and Stevenson, 1987; Ta and Faris, 1990); the use of ^{15}N-labelled compounds makes it possible to distinguish the behaviour of soil-native N from fertilizer and compost-derived N. However little information is available on soil nitrogen dynamics after adding compost; recently Kirchmann (1990), has studied N transformations in soil treated with ^{15}N-labelled fresh and composted cow manure, reporting a slow N release from the composted material. The composting process of organic residues sequesters N into more stable compounds and changes the amount of plant-available N (Hammouda and Adams, 1987). The aim of our experiment was to compare the N partitioning in soil after incorporation of ^{15}N-labelled dried or composted ryegrass during five months of incubation in controlled experimental conditions. Cellulose was added to dried ryegrass so as to give it a C/N ratio similar to that of composted ryegrass.

Materials and methods

Soil

Cultivated sandy soil was sampled from the subsuperficial (20–40 cm) layer, air-dried, sieved (<0.71 mm) and analysed prior to experiment. The soil had the following characteristics: pH (H_2O) 6.4; total N 0.8 g kg^{-1}; organic C 5 g kg^{-1}; total P 4.4 g kg^{-1}; CEC 7.9 cmol kg^{-1}; sand 74.6%; silt 20.4%; clay 5%.

Production of ^{15}N-enriched plant material

The labelling of plant material with ^{15}N was accomplished by growing ryegrass (*Lolium italicum L.*) with a modified Hoagland and Arnon nutrient solution including tagged $Ca(NO_3)_2$ (11.5 atom% ^{15}N) as the only N source. The N contained in the nutrient solution was almost completely recovered by plants. Plants were harvested when the leaf tip began to scorch, oven dried at 40°C and analysed. ^{15}N enrichment of roots and shoots was about 9.96% ± 0.95: thus only about 1.5% of the N taken up by plants was derived from unlabelled sources (see reserves, water and air).

Production of the labelled compost

Chopped ryegrass (100 g) was mixed with 96 g of thin stripped laboratory paper (pure cellulose) to increase the C/N ratio (Table 1), moistened to 50% moisture level and transfered to a closed micro-plant, with both air inlet and outlet checked. Since the composting process must be carried out in aerobic conditions, air washed with 0.1 *N* H_2SO_4 and moistened with distilled water was blown into the composting material. At the beginning of composting the temperature was 30°C; during the first 15 days it was artificially and gradually raised to 50°C and maintained constant until the end of the process (60 d). The compost was oven dried at 40°C, weighed, finely ground and analysed (Table 2); the pH, C/N ratio, CEC values were similar to those of a wide range of commercial composts (Jacas et al., 1987; Zorzi and Urbini, 1989); the % ^{15}N was 9.30 ± 0.80.

Table 1. Carbon and nitrogen contents of organic materials (g)

	Dry weight	Carbon	Nitrogen	C/N	% ^{15}N
Ryegrass	100.00	37.38	3.96	9.56	9.96
Cellulose	93.00	37.20	0.037	1000.00	
Composted ryegrass	96.31	36.50	2.52	14.48	9.30

Incubation experiment

Twenty jars (250 mL) were filled with air-dried soil (150 g); then the soil was amended with either dried ryegrass (R-treatment) or composted ryegrass (C-treatment) at an application rate of 20 tons of dry matter per ha (7.3 t of C ha^{-1} and 504 kg of N ha^{-1}). Therefore, in the C-treatment we applied 0.75 g of composted ryegrass per 150 g of dry weight soil while in the R-treatment we applied 0.43 g of dried ryegrass and 0.31 g of cellulose per 150 g of dry weight soil.

The amended soil in each jar was moistened to 50% of the water-holding capacity and dark incubated at 25°C with water content readjusted each day; at different incubation times (0–1–2–3–5–8–12–16–20 weeks) one jar for each treat-

Table 2. Chemical characterization of the composted ryegrass (on a dry matter basis)

Organic C	365.0 g kg^{-1}
Humic acid C	27.9 g kg^{-1}
Fulvic acid C	27.7 g kg^{-1}
Humine C	290.0 g kg^{-1}
Organic matter	629.3 g kg^{-1}
Ash	199.9 g kg^{-1}
C.E.C.	43.2 cmol kg^{-1}
pH	8.3
C/N	14.5

ment was removed and different N forms were extracted from fresh soil. The amount of amended soil in each jar was enough to guarantee duplicate samples for each nitrogen determination.

KCl-soluble NO_3^- and NH_4^+ were determined according to Keeney and Nelson (1982), HCl-hydrolyzable forms (total N, NH_4^+, amino-acid N, amino-sugar N) according to Stevenson (1982), total N according to Bremner and Mulvaney (1982), NaOH-soluble and insoluble N according to Schnitzer (1982); values of HCl-hydrolyzable NH_4^+ were reported after subtraction of KCl-soluble NH_4^+; NaOH-soluble N was determined after dialysis with 1000 D membrane. Microbial biomass-N was determined by the fumigation-extraction method (Ocio and Brookes, 1990).

Each N form was transformed to NH_4^+ and steam-distilled. Two distillations were carried out for each N form: only the second one was used for isotopic analysis. Moreover, water and ethanol were distilled between different samples to prevent cross contamination.

After steam-distillation samples were acidified by adding a few drops of H_2SO_4 (1:4), evaporated to dryness, redissolved in ethanol three times to exclude boric acid and then redissolved in water to provide an N concentration of ca 20 $\mu g\ mL^{-1}$. Gas samples for ^{15}N determination by emission spectrometry (Jasco 150 Analyzer) were prepared according to Nannipieri et al., (1985).

All the results have been expressed on an oven-dry soil basis (105°C) and are the means of four replications.

The recovery of dried and composted ryegrass-derived N (labelled N) was calculated as follows (He et al., 1988): labelled N = A (X − C)/(Y − C) where A is the total N in each fraction, X is the % ^{15}N of that fraction, Y is the % ^{15}N of applied dried or composted ryegrass and C is the % ^{15}N of native soil N.

Data were statistically analysed following standard procedures for analysis of variance (ANOVA) and differences between the means were tested by LSD test, using the SPSS system (SPSS Inc., Chicago, USA); reported differences were significant at $p < 0.05$.

Results and discussion

The composting process modified N distribution in plant material inducing an increase in the N of the higher molecular weight fractions such as the HCl-unhydrolyzable N, NaOH-soluble and dialyzed N, NaOH-insoluble N, while the most unstable HCl-hydrolizable N decreased (Table 3).

A high NO_3^- content was observed in the dried ryegrass probably as the result of plant growth under high nutrient conditions (hydroponic solution containing NO_3^-).

Application of the two amendments had a

Table 3. Distribution of nitrogen in different forms extracted from either dried or composted ryegrass

	g of N per 100 g of d.w. material		% of total N present in each fraction	
	Ryegrass	Compost	Ryegrass	Compost
Total N		2.52	100.00	100.00
HCl-hydrolyzable forms:				
Total N		1.88	82.07	74.60
Amino-acid N		0.78	32.07	30.95
Ammonia N		0.29	19.70	11.51
Amino-sugar N		0.08	3.79	3.17
Hydrolyzable unknown N		0.73	26.51	28.97
NaOH-soluble N		0.71	18.69	28.17
NaOH-insoluble N		1.33	17.17	52.78
KCl-soluble NH_4^+		0.009	2.37	0.36
KCl-soluble NO_3^-		0.19	28.03	7.78

significant effect on soil nitrogen availability (Figs. 1 and 2).

The concentration of total and labelled NH_4^+ in R-treatment markedly increased during the first 5 weeks; thereafter, the increase in exchangeable NH_4^+ was more gradual and lasted up to the 20th week. The initial increase may probably be due to microbial mineralization of plant-derived organic N; thereafter, mineralization of microbially immobilized native and/or labelled NH_4^+ cannot be excluded and it may simultaneously occur with the mineralization of plant organic N.

In the presence of composted ryegrass, the

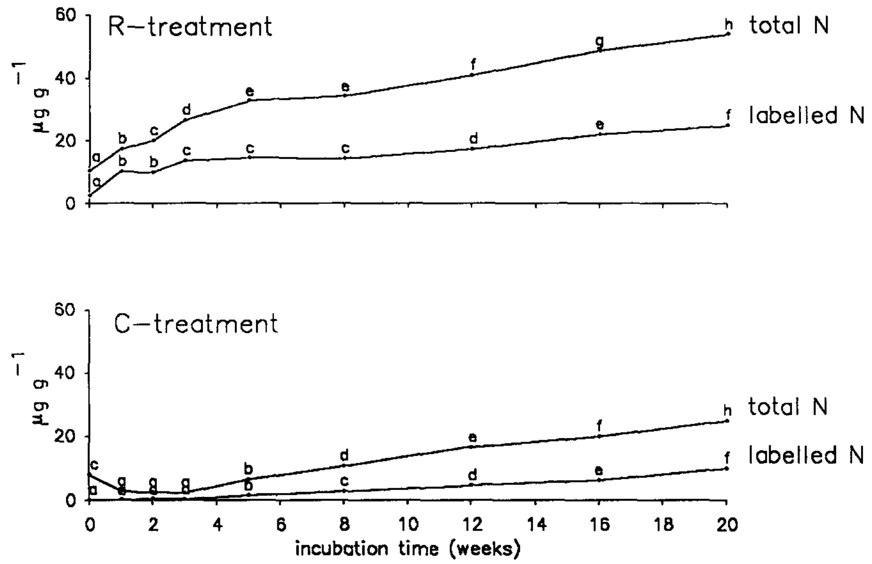

Fig. 1. Changes in exchangeable NH_4^+ (total and labelled) during incubation with dried (R-treatment) and composted (C-treatment) ryegrass. Same letters within each line indicate no significant differences between time.

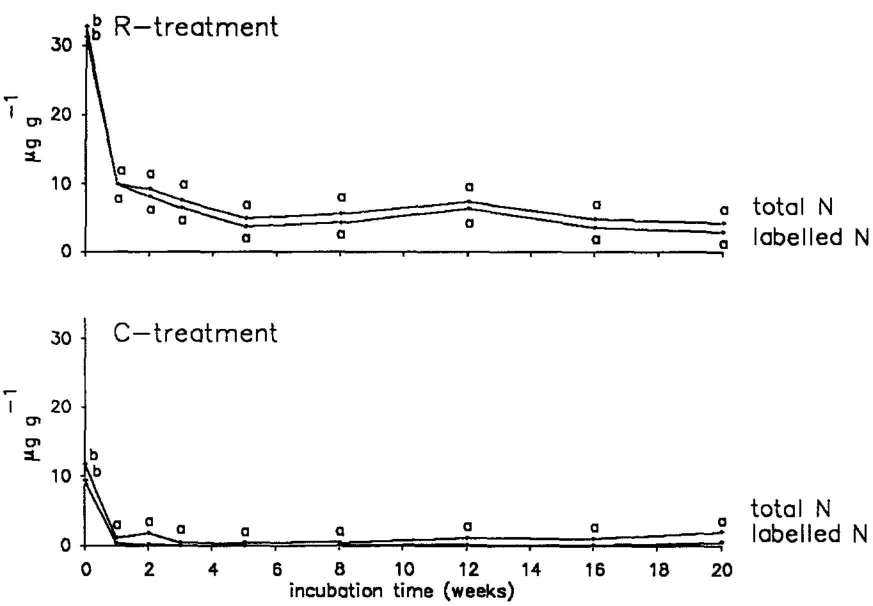

Fig. 2. Changes in NO_3^- (total and labelled) during incubation with dried (R-treatment) and composted (C-treatment) ryegrass. See note to Figure 1.

inorganic NH_4^+ amount decreased quickly at the beginning of the incubation as a result of the soil-native NH_4^+ immobilization. At the same time, the concentration of labelled NH_4^+ slowly increased, indicating a net mineralization of compost-derived N. After the 3rd week an increase in total NH_4^+ in soil occurred as a result of net mineralization of native N and labelled N.

The different behaviour of exchangeable $^{15}NH_4^+$ in the two treatments reflects the different net mineralization rates of organic labelled N compounds, probably as a result of different resistance to microbial attack.

At the beginning of the incubation time, soil-nitrate concentration was higher in the R-treatment than in the C-treatment (Fig. 2); nitrate concentrations rapidly decreased during the first week and reached very low values especially in the C-treatment after 2 weeks. Total N values remained constant between week 0 and 20 ($0,94 \pm 0.02$ mg g^{-1}), suggesting that the nitrate was converted to organic N by microbes rather than lost as gaseous N (N_2 and N_2O) through denitrification.

The difference between the values for microbial N confirmed the different decomposition rates of the two treatments: in the C-treatment after 1 week of incubation, microbial N was 24 μg g^{-1}, while it reached 36 μg g^{-1} (+35%) in the R-treatment. At the end of the experiment, microbial N values were approximately the same in both treatments (22 μg g^{-1}) as a result of stabilization in microbial populations.

The results concerning N distribution in HCl-hydrolizable compounds are shown in Figures 3, 4, and 5.

During both treatments the amount of total and labelled HCl-hydrolizable N was not significantly different, except for the first two weeks where the net increase in HCl-hydrolizable N was equal to the decrease of NO_3^- (Fig. 3).

The amounts of NH_4^+ in the HCl-hydrolyzable fraction decreased during the first 3 weeks in both treatments and then remained almost constant (Fig. 4). The decrease of labelled N was greater in the R-treatment than in the C-treatment, probably as a result of microbial breakdown of easily degradable compounds accompanied by release of exchangeable NH_4^+.

The changes in HCl-hydrolizable NH_4^+ (valued after subtraction of KCl-soluble NH_4^+) were the inverse of changes in exchangeable NH_4^+, indicating the strict relation between these two

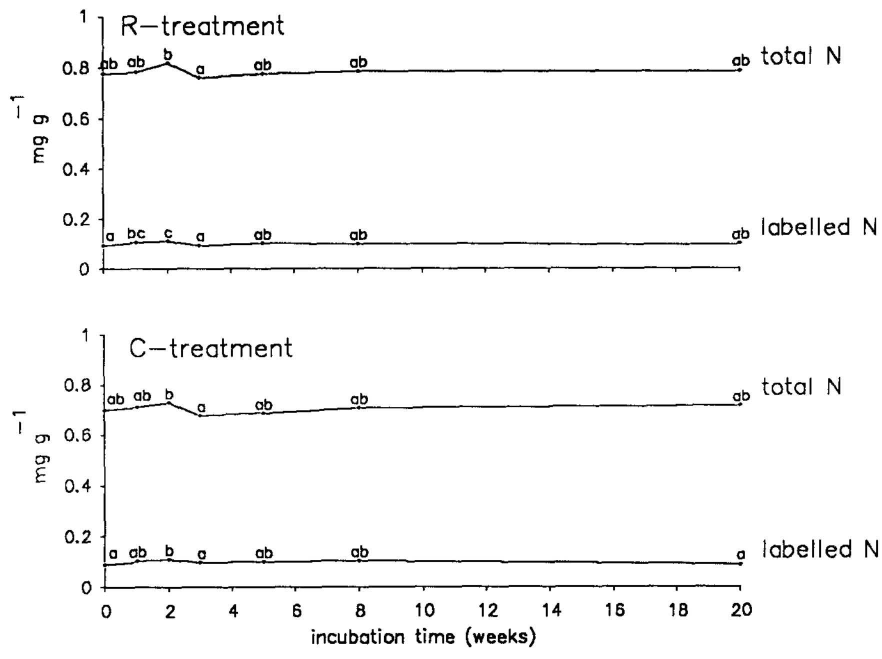

Fig. 3. Changes in HCl-hydrolyzable total N (total and labelled) during incubation with dried (R-treatment) and composted (C-treatment) ryegrass. See note to Figure 1.

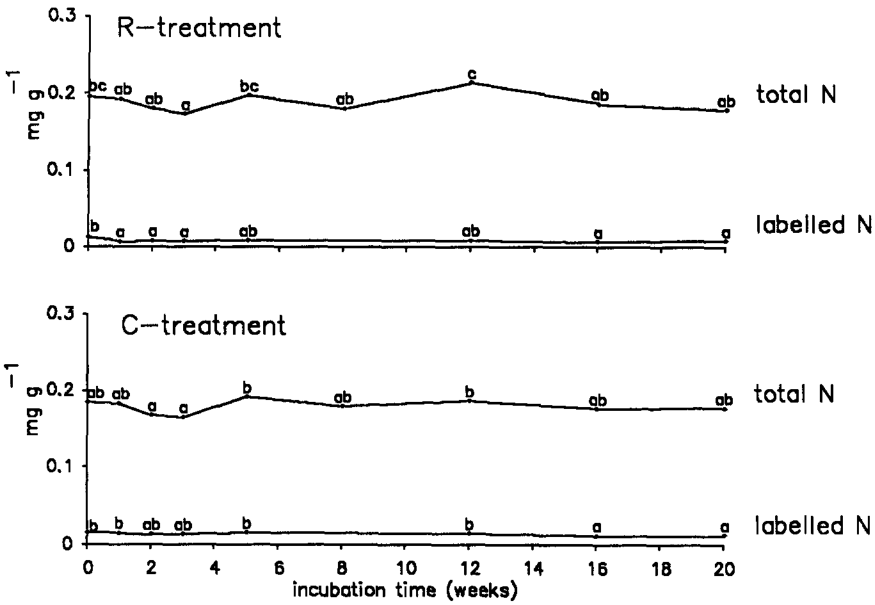

Fig. 4. Changes in HCl-hydrolyzable NH_4^+ (total and labelled) during incubation with dried (R-treatment) and composted (C-treatment) ryegrass. See note to Figure 1.

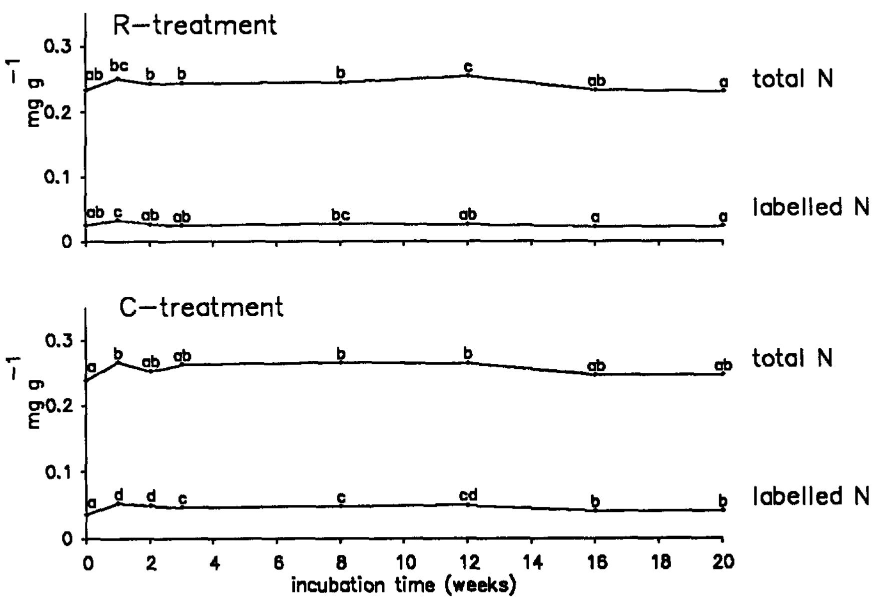

Fig. 5. Changes in HCl-hydrolyzable amino-acid N (total and labelled) during incubation with dried (R-treatment) and composted (C-treatment) ryegrass. See note to Figure 1.

pools, which was confirmed by the negative correlation chiefly for labelled N (C-treatment $r = -0.68$; R-treatment $r = -0.56$).

The amounts of HCl-hydrolizable amino-acid N slightly increased in both treatments after the first week and then remained stable for the rest of the incubation time. The labelled amino-acid N in the R-treatment showed a slight initial increase due to immobilization of non-amino-acidic plant-derived N followed by a decrease (Fig. 5); in the C-treatment a significant increase was observed during the first week, and thereafter values decreased more slightly remaining higher than the initial one. It may be hypothesized that amino-acids in composted ryegrass are bound to microbially recalcitrant humic compounds, while those of ryegrass are more easily degraded.

Total amino-sugar N did not show any significant difference in both treatments throughout the incubation. The labelled amino-sugar N increased in the R-treatment throughout, which can be seen better from the atom % ^{15}N values (Table 4). This finding is explained by the enhanced microbial growth due to dried ryegrass.

To further study the effect of composting on humification in soil, alkali-soluble N was monitored. In both treatments the amount of humic N did not change significantly during the whole experimental period; nevertheless in the C-treatment values were always higher than in the R-treatment, indicating that humic substances derived from composted ryegrass were not quickly decomposed in soil.

Table 4. Atom % ^{15}N in amino-sugar fraction

	Weeks			
	0	2	8	20
R-treatment	0.98	1.36	1.46	1.79
C-treatment	1.00	1.05	1.02	1.24

In both treatments total NaOH-insoluble N increased up to the 8th week, particularly in the R-treatment (Fig. 6), and thereafter it remained constant. Moreover in the R-treatment there was an increase of labelled N, while the C-treatment showed an early drop followed by an increase. Because soil biomass largely consists of fungal tissue, which is mainly insoluble in alkali (Russel et al., 1983) these findings agree with the increase in microbial biomass N and with the changes in inorganic N (chiefly NO_3^-) also observed by He et al. (1988).

Recovery of ^{15}N and percentual distribution of N in the different forms (Table 5) showed that composting reduced N mineralization, because the percentage of total N as exchangeable NH_4^+ was lower in the C than in the R-treatment at the end of incubation. This was also confirmed by the smaller percentage decrease of HCl-hydrolizable total N observed in the C-treatment. At the same time we observed a net NO_3^- immobilization in both treatments, thus confirming other findings (Voroney and Paul, 1984); the large assimilation of nitrate in the R-treatment was probably due to the cellulose addition, also reported by Azam et al. (1988). Nitrate im-

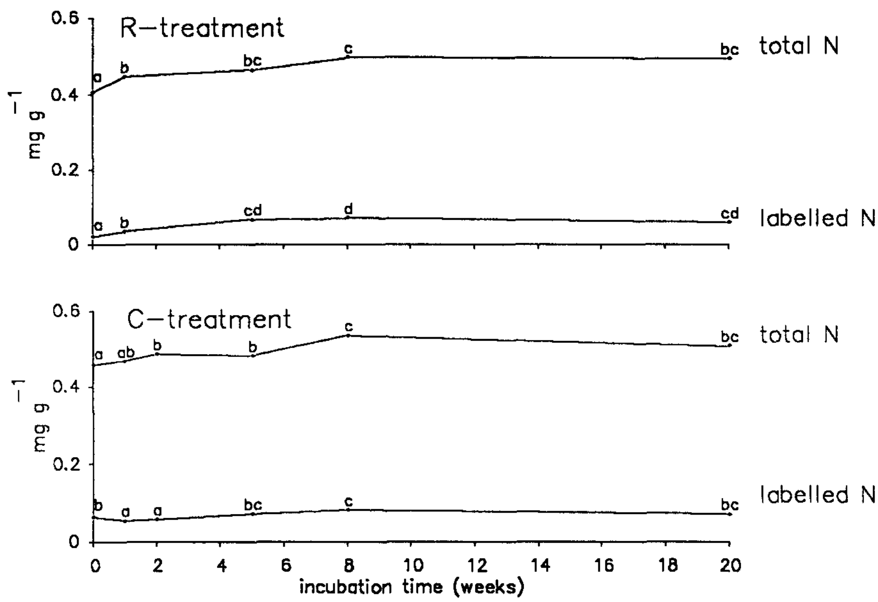

Fig. 6. Changes in NaOH-insoluble N (total and labelled) during incubation with dried (R-treatment) and composted (C-treatment) ryegrass. See note to Figure 1.

Table 5. Distribution in different forms of labelled N and total N at the beginning and at the end of incubation time

	% of total labelled N				% of total N			
	R-treatment		C-treatment		R-treatment		C-treatment	
	0 wk	20 wk	0 wk	20 wk	0 wk	20 wk	0 wk	20 wk
KCl-soluble NO_3	28.36	2.53	7.73	0.40	3.33	0.45	1.25	0.20
KCl-soluble NH_4	2.20	21.73	0.12	8.23	1.12	5.80	0.85	2.67
HCl-hydrolizable forms:								
Total N (excluded KCl-soluble NH4)	80.97	84.71	73.81	73.74	78.78	62.97	73.70	65.51
Ammonia N	17.20	11.21	12.01	9.53	20.86	19.13	19.75	18.93
Amino-acid N	30.93	28.77	28.75	32.97	25.02	24.74	25.42	26.30
Amino-sugar N	4.34	10.25	3.98	7.54	7.72	8.55	7.72	8.34
NaOH-soluble N	20.67	19.90	25.19	25.27	31.39	31.73	32.10	32.18
NaOH-insoluble N	14.77	42.49	51.62	57.41	43.48	52.75	48.76	54.22

mobilization may also account for the high level of NaOH-insoluble N, especially labelled N, found at the end of the incubation in the R-treatment. This agrees with the observation that when dried ryegrass was applied without other carbon sources, NaOH-insoluble labelled N did not increase to the same extent (unpublished data).

However, recent studies by Rice and Tiedje (1989) and Recous et al. (1990) have shown inhibition of nitrate assimilation by ammonium, even if it is present at low concentrations. We did not observe this, although ammonium levels were different in the two treatments, and always higher than the supposed inhibitory concentration. This could have been due to the different location of mineralization and immobilization processes, because they can be physically and/or temporally separated, as hypothesized by Drury et al. (1991). Furthermore, in our experimental design, the added organic matter was located in spotted microsites characterized by a high density of decaying microorganisms (releasing NH_4^+) while NO_3^- was present in the soil solution.

In the C-treatment the increase of labelled N in the amino-acid fraction is of particular interest: amino-acids in compost are probably present in compounds resistant to microbial decomposition. In addition, new amino-acids are synthesized from compost-derived N, whereas dried ryegrass amino-acids seem to be easily decomposed. Composting increased the resistance to microbial degradation of HCl-hydrolyzable N even though we did not observe any effect on humification.

Our results indicate that composting of plant material modifies N transformation by soil microorganisms; the immobilization of exchangeable NH_4^+ is particularly affected when soil is treated with recalcitrant organic compounds. The inorganic N released during mineralization of dried ryegrass exceeds the need of microbial biomass for N.

The release of exchangeable NH_4^+ occurring after five months incubation was estimated to be about 90 kg ha^{-1} from composted ryegrass (18% of total applied N) and about 195 kg ha^{-1} from dried ryegrass (39% of total applied N). Thus composted plant material releases less inorganic N than non-composted material, so reducing the risk of N losses.

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