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Disproportionately high N-mineralisation rates from green manures at low temperatures – implications for modeling and management in cool temperate agro-ecosystems

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Abstract

We examined the decomposition of *Medicago lupulina*, *Melilotus alba* and *Poa pratensis* at 3, 9, and 25 °C during 4 weeks. There was a strong temperature effect on the rate of CO₂ evolution, and thus the extent of energy exhaustion from the added substrates. However, there was no concomitant retardation of N mineralisation at low temperatures. In the analysis of variance of mineralized N the residue type gave a 10 times larger contribution to the regression than the temperature (T), whereas for CO₂ evolution residue type and temperature were equally important contributors. This indicates that although the temperature has a statistically significant effect on N-mineralisation it is substantially less than compared with the effect on carbon mineralisation in the materials examined. The retardation of carbon mineralisation was least strong in *Melilotus alba* that had a relatively low cellulose content, and a higher content of low molecular compounds. Though more research will be necessary to consolidate and explain this phenomena, it is likely that an important factor is a decrease in the bioavailability of C-rich polymers at low temperatures, and thus a preferential utilization of N-rich low molecular substances. Nitrification was not effectively deterred at 3 °C. Thus, in terms of management, it is pertinent to reconsider the timing of green manure and catch crop incorporation in cool temperate climate regions, since the rapid release of nitrogen, coupled with the relatively undeterred nitrification may result in a high N leaching risk by early incorporation, but a low risk for N immobilization at late incorporation, if N rich residues are used.

Introduction

In cool temperate climate agro-ecosystems catch crops or green manures are often incorporated during late autumn or early spring, or killed by the winter climate while it is cold. It has been assumed that turnover and mineralisation would be very slow at such low temperatures. Thus the risk of losing N from these crops during the subsequent winter is assumed to be low, and a major concern would be whether mineralisation upon incorporation of the catch crops residues into a cold spring soil would be fast enough to be beneficial to the succeeding crop. Previous results with catch crops, e.g. Thorup-Kristensen (1994) have in-

dicated that when catch crops died off during winter most of the first year N-mineralisation had already occurred before early spring. This finding has been corroborated by Breland (1994) and Müller and Sundman (1988). When catch crops were incorporated in the early spring, the nitrogen mineralisation occurred very fast and the subsequent barley crop took up the N during its early growth (Thorup-Kristensen, 1994). Van Schöll et al. (1997) examined N mineralisation from shoot and root materials from green rye at 1, 5, 10 and 15 °C. They found that 20% of the added organic N was mineralized after 10 weeks at 1 °C, and 90% of this had been nitrified.

Widely accepted conceptual models of the nitrogen mineralisation derived from organic materials assume a direct link to the mineralisation of carbon during

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their decomposition. This is reflected in a number of computer simulation models, that describe decomposition as the 1st order kinetic decay of several discrete pools, having a constant C–N ratios (Hansen et al., 1990; Jenkinson et al., 1987; Parton et al., 1988). It follows that the temperature dependency of N mineralisation in such models is the same as that of C mineralisation. Thus neither the high winter mineralisation of N observed from the winter killed catch crops, nor the fast mineralisation from the spring incorporated catch crops could be simulated by the DAISY model (Thorup-Kristensen, 1995; Thorup-Kristensen and Nielsen, 1998).

Kirchbaum (1995) analyzed 11 studies of temperature effects on C mineralisation and 9 studies on N dynamics and found that the temperature sensitivity of Q_{10} for CO_2 evolution was close to 9 at 3 °C and around 1 at 35 °C. The likeness of Q_{10} for N dynamics was confirmed but Kirchbaum noted that the data for N dynamics appeared to have overall lower temperature sensitivities and had less increase in temperature sensitivity at low temperature than the data for CO_2 efflux. However, due to the degree of scatter in the compiled data it could not be stated with confidence that the two processes had different inherent temperature sensitivities in the low temperature range.

The concern about N-leaching losses in European agro-ecosystems has triggered responses in systems research. One such response is founded on the assumption that intensive production systems that leave large amounts of nitrogen in soil after harvest (e.g. shallow rooted vegetables) can be made environmentally more benign by including 'catch crops' that can take up excess nitrogen during the cool season, and transfer it to the following cash crop during the next growing season (Thorup-Kristensen, 1994, 1995). Similarly, it has been assumed that 'Organic' or 'Biological agriculture' production systems can be improved by including nitrogen fixing crops in the cool 'autumn and winter' season (e.g. undersown with cereals). This presupposes that such organic resources (catch crops or N-fixing legumes) can effectively deliver the assimilated N to the following crop, so that actual loss reductions and fertility improvements are attained. Thus, it becomes crucial to gain a better understanding of the issue of N-mineralisation at low temperatures, since a synchrony between the delivery of N from green manures and the demand of N from subsequent crops is desirable and sometimes essential to minimize losses.

Based on the literature, it was apparent that controlled laboratory studies of green manure decomposition at varying and low temperatures were needed. We hypothesized that N from green plant materials could mineralize rapidly at low temperatures, where C mineralisation is depressed. The basis for this hypothesis was mainly rooted in our experience from field studies, but also based on observations of Nicolardot et al. (1994) that mineralisation of low molecular carbon substances (sucrose) were less affected by low temperature than high molecular (holocellulose). The following study represents the first trial that will be supplemented by more detailed examinations in the future.

Materials and methods

Experimental design

The experiments were conducted using a loamy sand (11% clay, 34% silt, 55% sand), containing 2% organic matter, sampled from the plow layer of an arable soil from research center Aarslev, Denmark. The soil was initially air-dried and sieved at 2 mm, then moistened to 60% of WHC and pre-incubated for 3 weeks in the dark at 14 °C.

The experimental design included additions of *Melilotus alba*, *Medicago lupulina* and *Poa pratensis*. Characteristics of the added plant materials are given in Table 1. A control without additions of plant material served as reference. Air dried plant material was chopped to ca. 15–20 mm lengths and mixed into portions of 340 g soil (DM) to a concentration of 4.0 g plant material (DM) kg^{-1} soil, corresponding to 10 ton green manure ha^{-1} to a depth of 15 cm. The green manure residues were extracted from soil samples (0–20 cm) collected in spring before incorporation. The extraction was done by a manual dry removing of visible plant residues (leaves, stems and roots). The plant material was then subdivided in a fine and a coarse fraction. For this study, we used the coarse fraction only. This extraction procedure was chosen in order to retain water soluble components in the plant residues which are assumed to be easily decomposable.

The soil samples were placed in triplicate 3 L jars containing a CO_2 trap (20 ml 2M NaOH), and a beaker containing water to maintain a water saturated atmosphere. Five jars containing only the CO_2 -traps and water served as blanks. The soils were then incubated in the dark at 3, 9 and 25 °C. For the 3 and 25 °C

Table 1. Biochemical quality characteristics of the plant materials

	C	N	ash	cellulose %	lignin	C-N	lignin-N ratio
<i>Poa pratensis</i>	45	2.0	6.2	32	5.4	22	2.7
<i>Medicago lupulina</i>	35	2.2	13.6	31	10.0	15.4	4.5
<i>Melilotus alba</i>	41	3.2	9.8	21	5.1	12.7	1.6

experiments, CO₂ was sampled at day 2, 3, 4, 8, 14, 22 and 36, while NH₄, NO₃ and soil microbial biomass N was determined at day 0, 3, 8, 22 and 36, using duplicate sub-samples of 20 g soil from each jar. During the course of the experiment the water content in the soils was monitored, but no adjustments were necessary. Since the 9 °C experiment was initiated earlier, as part of another investigation a somewhat different time schedule was used: CO₂ was sampled at day 1, 3, 4, 7, 11, 17, 24, 30, 36, 43 and 51, while N_{min} was determined at day 0, 1, 3, 7, 17 and 51, whereas soil microbial biomass N was not determined. From day 12 to 14, the temperature rose gradually (average 12.9 °C) due to a fault in the electronic temperature control of the 9 °C chamber. This was corrected after day 14, and the data are presented in this paper as 9 °C data, since this has caused only minor disturbance.

Chemical analysis

Soil respiration was monitored by measuring the CO₂ trapped in the NaOH solutions. The bicarbonate and carbonate ions in the NaOH solutions were precipitated with excess BaCl₂. Total CO₂ was determined by titrating the remaining NaOH with HCl. Mineral N (ammonium and nitrate) was measured in 0.5 m K₂SO₄ extracts by standard colorimetric methods using flow-injection analysis (Keeney, 1982). Cellulose and lignin content of plant materials was analysed by the method of Goering and Van Soest (1970).

Data analysis

The 3 and 25 °C data were collected at Frederiksberg on the same dates by the same operator, whereas the 9 °C data were collected at Taastrup at a different time, with different time intervals and with greater operator variability. Therefore only the 3 and 25 °C data were subject to detailed analysis of variance using the SAS General Linear Model's procedure. The 9 °C data have been excluded from the overall analysis of variance due to their different origin and quality, but they have

been included in the figures, since they supplement and support the main dataset.

Results and discussion

Temperature effects on CO₂ respiration

For all materials, there was a distinct effect of increasing temperature on carbon mineralisation. At 25 °C a plateau was approached rapidly during the first 4 days of incubation and leveling off during the remainder of the period (Figure 1). At 9 °C, the same extent of decomposition was attained towards the end of the experiment, but during the first 4 days the course of respiration was identical to that in the 3 °C experiment. Thereafter, respiration increased to the level of that at 25 °C. At 3 °C carbon mineralisation was substantially retarded throughout the incubation period.

For *Poa pratensis* and *Medicago lupulina* ca. 30% of the added carbon was respired as CO₂ by the end of the experiment at 9 and 25 °C, whereas at 3 °C less than 10% was liberated (Table 2). In contrast to this, more than 40% of added carbon respired at 9 and 25 °C in the *Melilotus alba* treatment, and similarly at 3 °C the respiration approached 20% of the added carbon. This indicates a clear difference in substrate quality between the materials. The biochemical quality characteristics (Table 1) shows that the *Melilotus alba* material had a high concentration of N and a lower concentration of cellulose (acid hydrolysable), indicating that low molecular weight compounds were abundant in this material. This assertion was supported by the observation that even at 3 °C almost 10% of added carbon was respired after 4 days of incubation from this treatment (Figure 1).

In the analysis of variance (Table 3) the temperature (T) gave a higher contribution to the regression than the residue type, even though the control treatment (no material added) was included. Since the

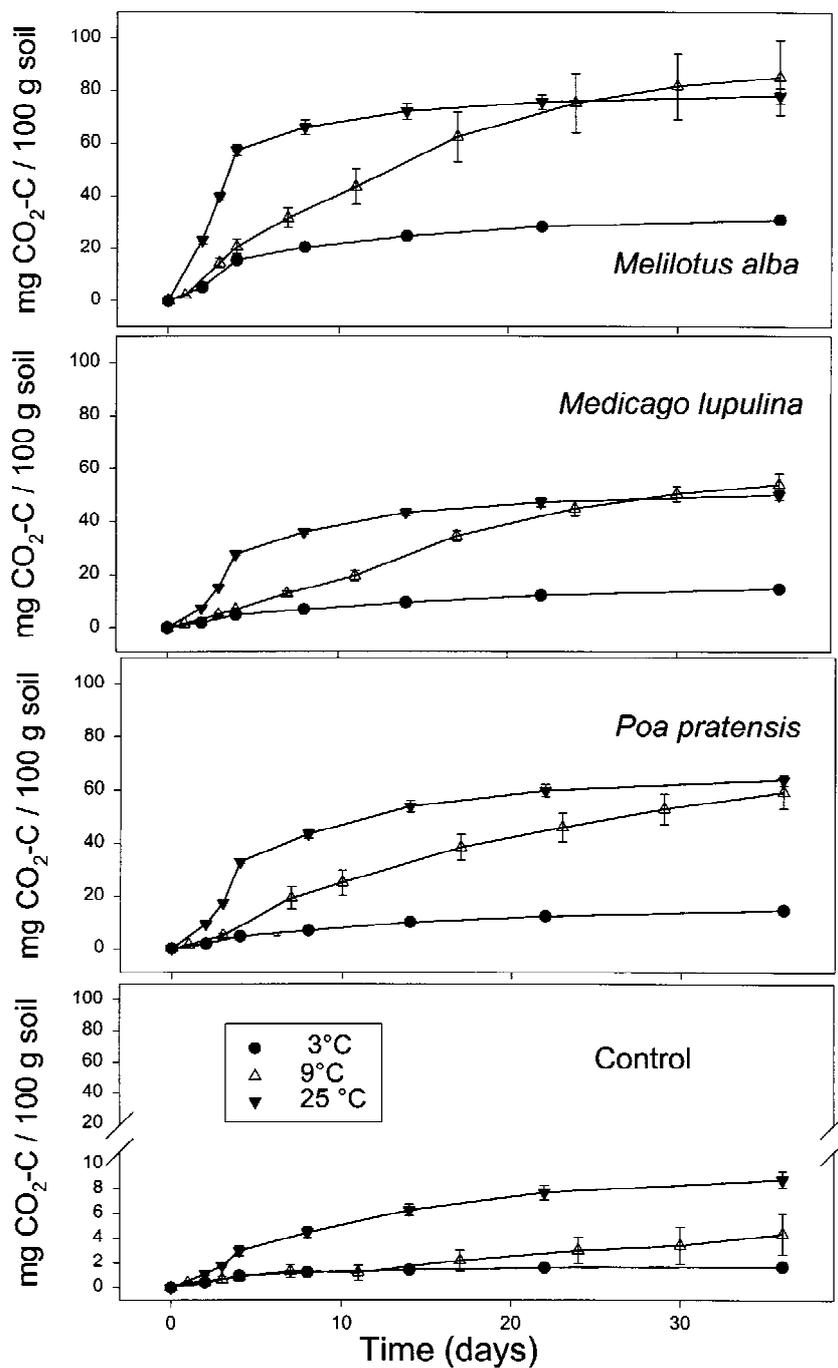


Figure 1. Cumulative CO₂ evolution with time (standard error drawn as bars) from *Poa pratensis*, *Medicago lupulina*, *Melilotus alba* and control soil.

Table 2. The CO₂ and N mineralisation in% of added C and N, at the end of the experiment. The numbers have been calculated by subtracting mean values of control soil mineralisation and the standard error (in square brackets) based on the sum of squares of standard deviations from control and residue treatments

	N _{min} % of added N						CO ₂ % of added C					
	3 °C		9 °C		25 °C		3 °C		9 °C		25 °C	
<i>Melilotus Alba</i>	39.6	[2.3]	38.2	[2.7]	59.5	[14.2]	17.9	[0.2]	44.9	[3.9]	42.4	[1.2]
<i>Medicago Lupulina</i>	28.2	[2.0]	27.9	[2.0]	48.0	[10.9]	9.2	[0.3]	28.7	[1.2]	29.5	[0.9]
<i>Poa Pratensis</i>	25.6	[2.6]	6.9	[3.8]	9.1	[6.3]	7.2	[0.2]	28.9	[1.7]	30.5	[0.8]

Table 3. The relative contribution to total variance of the main effects in the combined 3 and 25 ° data. Unless explicitly stated the results were significant ($p < 0.0001$)

Response variable	Residue	T	residue * T ^{b)}	time	error	Total
	type ^{a)}					
	% contribution to variance					
N _{min}	39.8	3.1	1.0 (n.s.)	29.3	26.7	100.0
CO ₂ respiration rate	12.3	14.2	4.3	46.2	23.0	100.0

^{a)}Includes *Melilotus alba*, *Medicago lupulina*, *Poa pratensis* and the Control treatment (no material added).

^{b)}The interaction between residue and temperature.

response variable tested was the CO₂ mineralisation rate a very high time dependency was to be expected and, therefore, the contribution to variance of both temperature and residue type appear relatively small.

Temperature effects on N mineralisation

Net N mineralisation from the plant materials (Figure 2) did not show a marked temperature dependency in line with that of the CO₂ respiration. At 3 °C, 25–40% of the added N was mineralized during 35 days, in the various materials (Table 2). Addition of the *Poa pratensis* material gave rise to a characteristic pattern of initial mineralisation and subsequent immobilization at 25 °C. This pattern was delayed at 9 °C but did not appear at 3 °C within the timeframe of the experiment. This could indicate a preferential substrate utilization at the low temperature, i.e. that low molecular weight compounds abundant in N were utilized, whereas cellulose and other C rich polymers were less available. A retardation in the decomposition of polymeric substances could be due to reduced microbial synthesis of extracellular enzymes at 3 °C, and a concomitant reduction of the faunal activity that by comminution exposes the interior tissues to enzymatic attack. Thus it is generally believed that fauna is rendered inactive at such low temperatures (Sulkava, 1996). For both *Medicago lupulina* and *Melilotus alba*

treatments, however, an immobilization phase was apparent at the end of the experiment at 3 °C, indicating that the activity of organisms decomposing energy rich compounds had increased.

In the analysis of variance (Table 3) the residue type gave a 10 times larger contribution to the regression than the temperature (T). This indicates that although the temperature has a significant effect on N-mineralisation it is substantially less than compared with the effect on carbon mineralisation in the materials examined, and thus supports the contention that that N-mineralisation rates are disproportionately high from green manures at low temperatures. This is despite that by comparing the CO₂ mineralisation rates (differentials) with the measured N_{min} values (that are essentially integrals) the analysis is strongly skewed towards finding a stronger temperature dependency in the N_{min} variance, since integrals include temperature effects in previous results.

From the measured data, it is apparent that the carbon mineralisation of *Melilotus alba* rich in low molecular substances was less affected by low temperatures than the other treatments. This is in general agreement with the observations of Nicolardot et al. (1994) on disproportional temperature effects on decay of holocellulose, relative to sucrose. Henriksen and Breland (1999) noted that N release during the first autumn and winter months from various crop

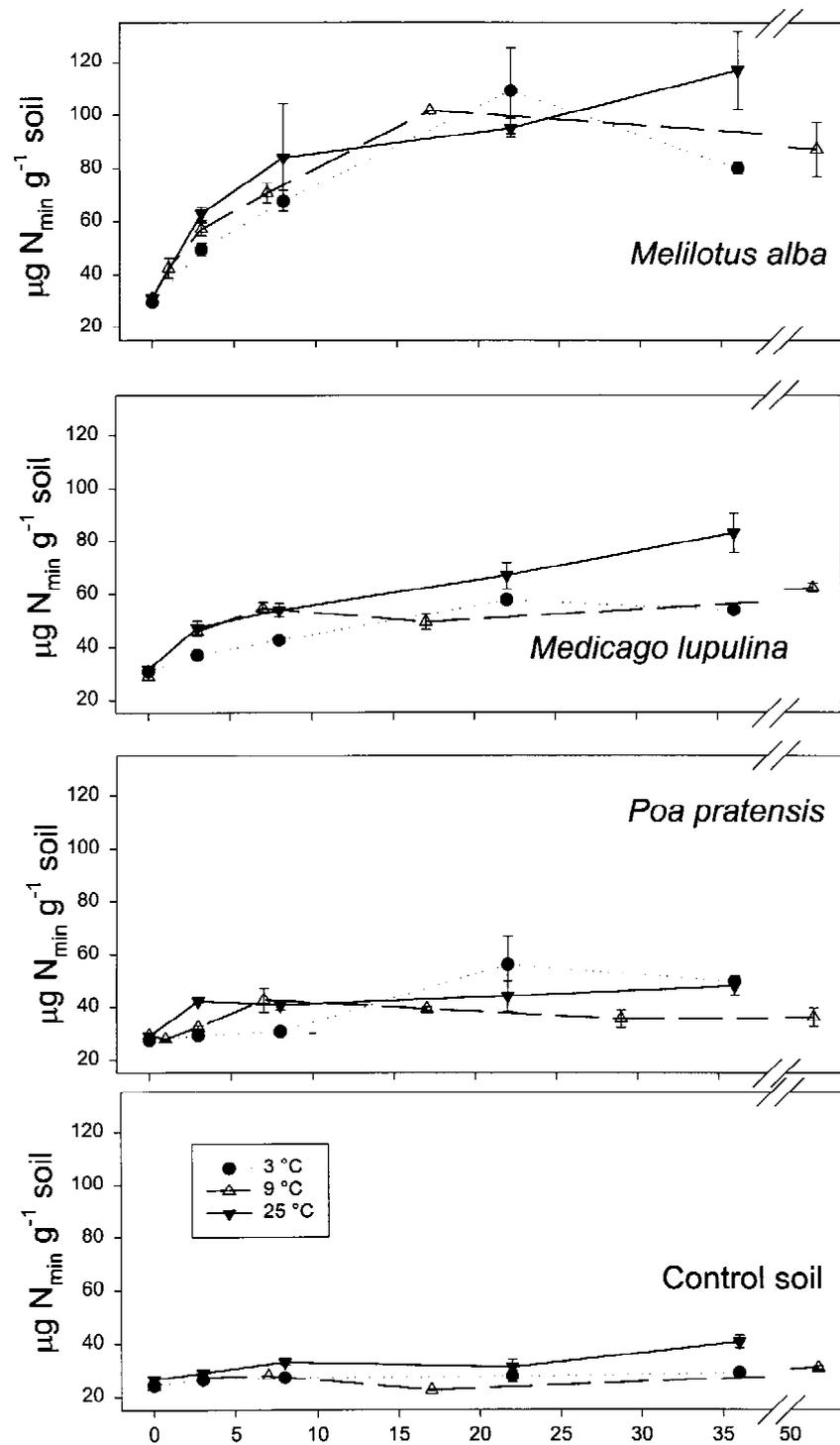


Figure 2. Net nitrogen ($\text{NH}_4 + \text{NO}_3$) mineralisation with time (standard error drawn as bars) *Poa pratensis*, *Medicago lupulina*, *Melilotus alba* and control soil.

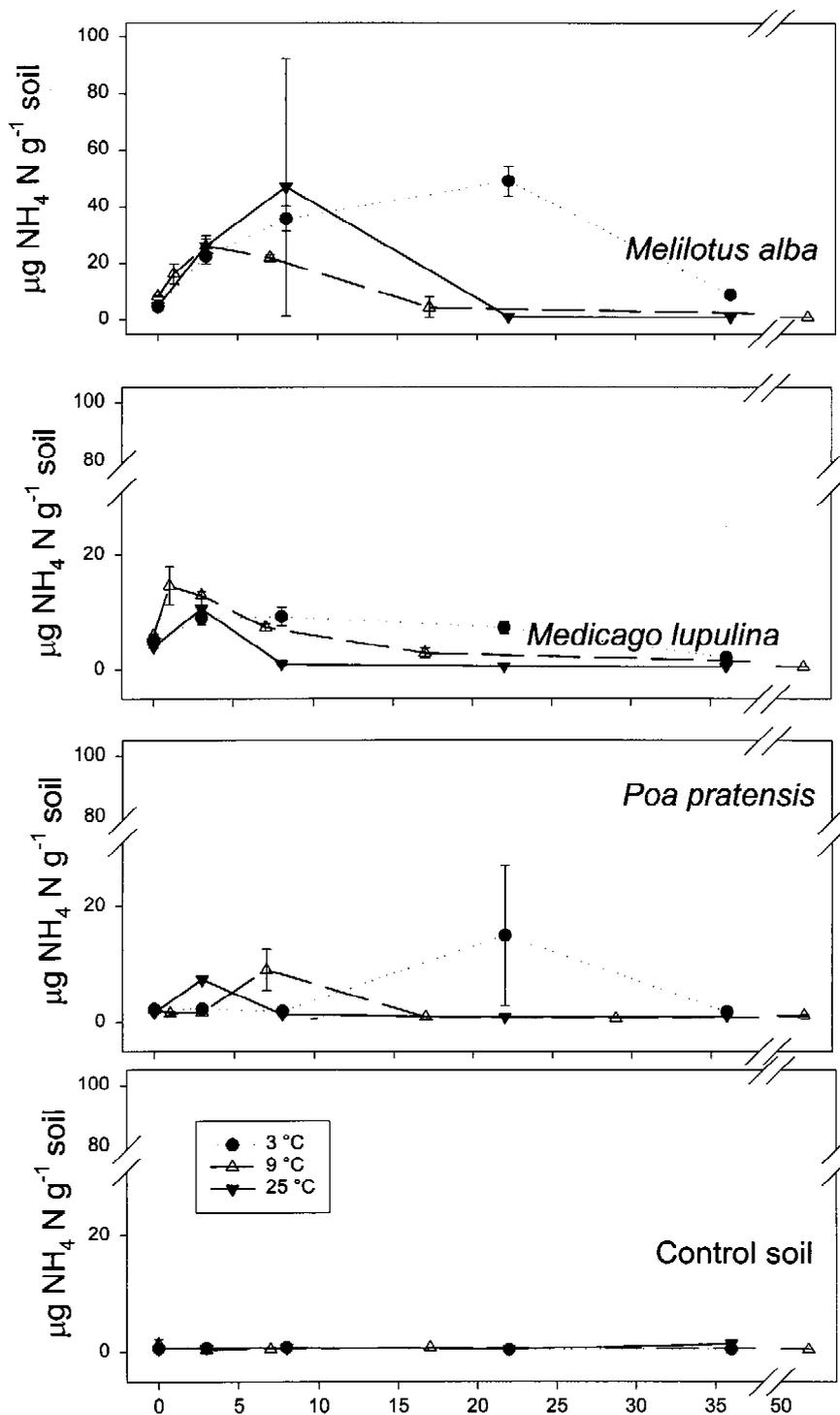


Figure 3. Concentrations of $\text{NH}_4\text{ N}$ in soil with time (standard error drawn as bars) from *Poa pratensis*, *Medicago lupulina*, *Melilotus alba* and control soil.

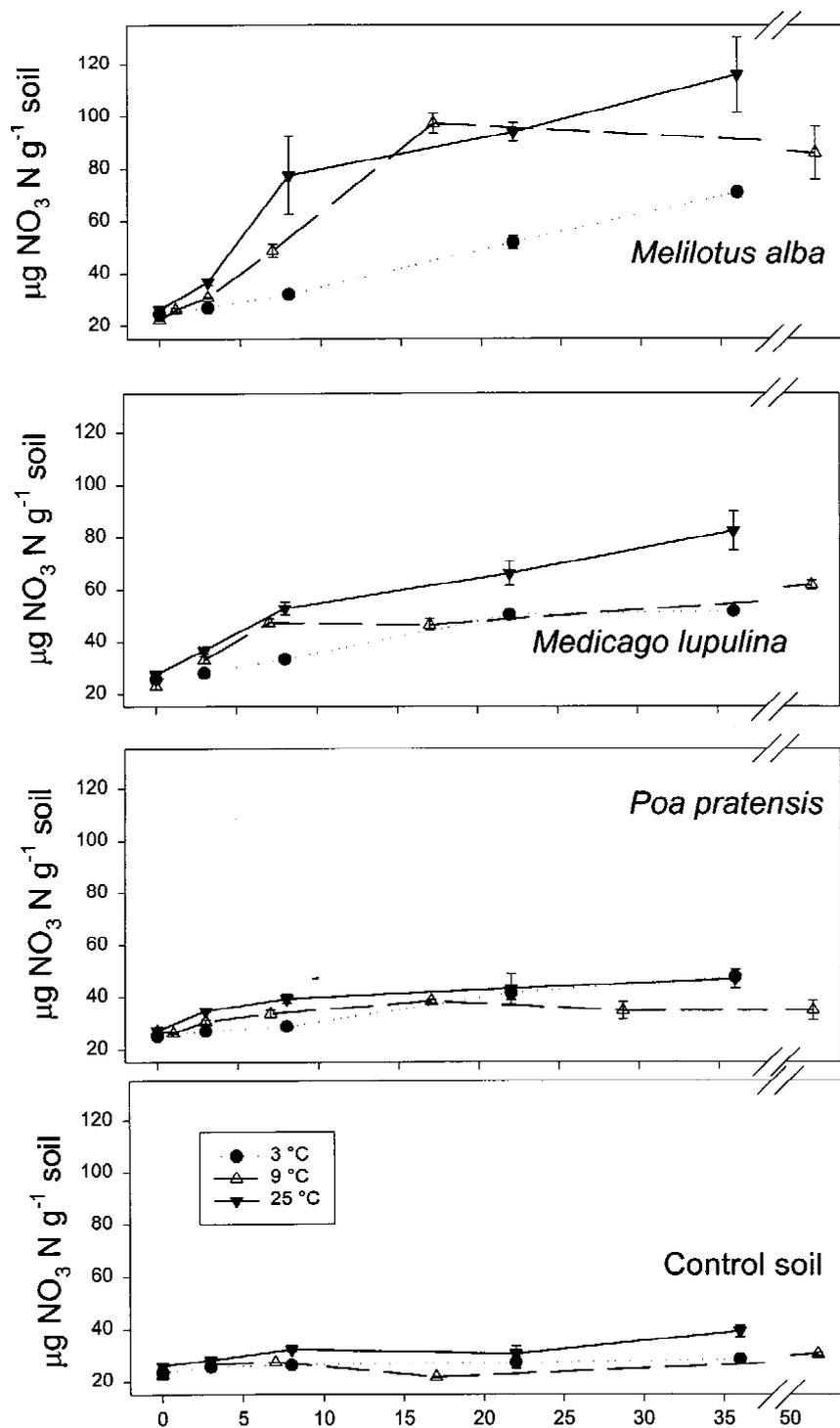


Figure 4. Concentrations of $\text{NO}_3 \text{ N}$ in soil with time (standard error drawn as bars) from *Poa pratensis*, *Medicago lupulina*, *Melilotus alba* and control soil.

residues was considerably underestimated by their model, and suggested that low temperatures restrict microbial N immobilization more than gross decomposition. Kirchbaum's (1995) observation that the data for N dynamics appeared to have overall lower temperature sensitivities and had less increase in temperature sensitivity at low temperature than the data for CO₂ efflux is supported by our observations.

Temperature effects on nitrification

Nitrification was expected to be severely restricted at the low temperature, but as seen in Figure 3 in both the *Medicago lupulina* and *Melilotus alba* treatments the disappearance of NH₄-N indicates that substantial nitrification occurred even at 3 °C. This is in agreement with the finding of Van Schöll et al. (1997). Thus although the nitrate production was lower at 3 °C (Figure 4) the nitrification rate was sufficient to increase nitrate contents in the treated soils 20–40 µg NO₃-N above the level of the control soil after 35 days. Considering that the amounts of residue applied (10 t residue DM ha⁻¹, to 15 cm depth) is within the agronomic range and that the above mentioned amounts of nitrate correspond to 50–100 kg N ha⁻¹ that can potentially be leached during the winter it seems pertinent to reconsider the timing of green manure and catch crop incorporation.

Implications for modeling

As stated in the 'Introduction', the models available are unable to describe the disproportionately high N mineralisation rates from green manures at low temperatures. To further include the N dynamics, it would be necessary to base both modeling and measurements on gross mineralisation processes, since net mineralisation is the end result of a largely unknown balance between gross immobilization and mineralisation. We hypothesize that the gross mineralisation of N-rich low molecular substrates was relatively less affected at low temperatures than gross immobilization, that would arise from the utilization of energy rich (high C–N ratio) polymers. The constraints for polymer decomposition at low temperatures could be caused by a decrease in both comminuting faunal activity and in the microbial synthesis of extracellular enzymes. Presumably this is the main reason for the measured net outcome of disproportionally high N-mineralisation at low temperatures.

Environmental impact and management implications

Our results confirm that N mineralisation from easily decomposable plant material can occur rapidly even in cold soil, as has been inferred from previous field studies (Breland, 1994; Müller and Sundman, 1988; Thorup-Kristensen, 1994). This has a number of consequences for the optimal management of green manure crops and catch crops in cool temperate climate regions. The rapid release of N means that it is very important not to incorporate the plant material too early, as leaching loss of mineralized N may then occur. This is exacerbated by the apparent substantial nitrification occurring even at 3 °C. On the other hand, for the materials used in this study, a late incorporation should allow sufficient time for N mineralisation to become available for the subsequent crop. However, it should be noted that the basis for making generalized statements is limited. Certain commonly used catch crops (e.g. *Lolium perenne*) develop high C–N ratio tissues even at cold temperatures and, therefore, such crops can cause substantial immobilization of N detrimental for the following crop, if they are ploughed under shortly before sowing.

Simulation models will have to be changed to be able to simulate the fast N mineralisation at low temperatures, otherwise they cannot make realistic simulations of the effects of green manures, catch crops or other situations where fresh green plant material is incorporated into cold soil.

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