Pergamon

PII: S0038-0717(95)00154-9

Soil Biol. Biochem. Vol. 28, No. 4/5, pp. 655–663, 1996

0038-0717/96 $15.00 + 0.00

NITROGEN MINERALIZATION AND MICROBIAL BIOMASS AS AFFECTED BY SOIL COMPACTION

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(Accepted 17 October 1995)

Abstract—Soil compaction may retard decomposition of organic matter and N mineralization and increase gaseous losses of N. We studied the effect of soil compaction on the turnover of N from added organic materials in pots with Italian-ryegrass (*Lolium multiflorum* Lam.) plants. Solid cattle manure or ^{15}N-labelled white-clover (*Trifolium repens* L.) material was incubated at controlled temperature (15°C) and moisture (pF 2.4 or pF 1.8) in a sandy loam with a bulk density of 1.1 or 1.4 g cm^{-3}. The distribution of labelled clover N was determined after 22, 42, 64 and 98 days. Also, net N mineralization from manure and clover was determined by subtraction of the values for unamended soil. Hydrogen sulphide, volatile fatty acids, soil acidity, phytotoxicity (bioassay), soil atmosphere composition (N_2O, O_2, CO_2), and colony-forming bacteria after anaerobic and aerobic incubation of dilution plates were determined as selected indicators of anaerobicity in the soil.

After 98 days at pF 2.4, soil compaction (1.4 g cm^{-3}) had reduced the net mineralization of clover ^{15}N by 18% compared to uncompacted soil, a reduction corresponding to 4% of added ^{15}N. Total ^{15}N recovery was not reduced by compaction, and there was no evidence of anaerobic metabolism. Consequently, increased gaseous N losses or retarded decomposition due to O_2 deficiency could not account for the difference. Compaction increased ^{15}N retention in soil organic matter by 8% and in microbial biomass (chloroform fumigation–extraction) by 1% of added ^{15}N. The compaction effects increased successively during the incubation. The negative effect of compaction on N mineralization was stronger at the higher soil moisture content (pF 1.8, sampled on day 64 only), but no evidence of anaerobicity was detected. Compaction effects on N mineralization, bacterial biomass (microscopy) and microbial biomass determined by difference (amended minus unamended soil) agreed with the ^{15}N results.

Soil compaction reduced the volume of pores with neck dia > 30 μm, i.e. pores available to nematodes, from 30.4 to 14.6% of total bulk volume. The volume of pores < 3 μm, i.e. pores that are unavailable to cellular organisms or available only to bacteria and fungi, increased from 12.7 to 15.6%. The results strongly suggest that N mineralization in compacted soil was reduced by increased physical protection of organic materials and microbial biomass against further attack, particularly by nematodes grazing on microorganisms. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Soil compaction is a common feature of modern agriculture, especially in humid climate, where crop yield and N uptake may be substantially reduced (Hansen, 1996). Deterioration of the plant root environment is an important reason for the negative effects, but a reduced decomposition of organic matter may also contribute.

Soil compaction reduces the total soil pore volume and changes the pore size distribution towards a higher percentage of small pores. In these pores, organic materials may be physically protected against microbial attack, and microorganisms may be inaccessible to predating protozoa and nematodes (Elliott and Coleman, 1988; van Veen and Kuikman, 1990). Hassink and co-workers found that soil N mineralization was negatively and microbial biomass positively correlated to the fraction of small pores (< 1.2 μm dia) in various soils, while the nematode biomass was positively correlated to the volume of pores between 30 and 90 μm dia (Hassink, 1992; Hassink *et al.*, 1993).

Reduction of the total soil pore volume after compaction also increases the probability of anaerobic conditions. This may strongly inhibit degradation of lignocellulose (Colberg, 1988), which has a major influence on the degradability of plant residues (Pinck *et al.*, 1950). Also, animal manure contains a substantial amount of lignin or lignin-like structures that have formed complexes with proteins (McCalla *et al.*, 1977). Gale and Gilmour (1988) observed that anaerobic conditions initially stimulated net N mineralization from plant residues. However, decomposition and N mineralization ceased when mainly recalcitrant residues remained. Gaseous losses of N by nitrification and denitrification will also

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increase because of impaired aeration (Hansen *et al.*, 1993).

Previous studies on soil compaction have dealt with carbon turnover, and the results were partly conflicting (van der Linden *et al.*, 1989; Kaiser *et al.*, 1991; Santruckova *et al.*, 1993). To our knowledge, no work has been done on the effect on N turnover. Nitrogen mineralization is more affected than carbon mineralization by physical protection (Hassink *et al.*, 1993; Breland, 1994). Consequently, we expected N mineralization to be a potentially more sensitive parameter.

Our objective was to study the effect of compaction on the fate of N from organic materials added to soil. We carried out a pot experiment under controlled temperature and moisture conditions with ^{15}N-labelled white-clover material and unlabelled cattle manure added to a sandy loam. We particularly focused on whether possible compaction effects could be explained by physical influences on microbial turnover or by impaired aeration.

MATERIALS AND METHODS

Experimental plan, pots, soil and establishment of plants

A pot experiment was carried out with treatments as listed in Fig. 1 and samplings on days 22, 42, 64 and 98 (three parallel pots). Pots with the lower soil moisture content (pF 2.4) were removed at all samplings, while pots with the higher moisture level (pF 1.8) were sampled on day 64 only.

The soil was a sieved (5-mm mesh) sandy loam (Typic Udorthents; USDA soil classification) with 3.3% organic matter, 0.09% total N, 5% clay (<2 μm), 7% silt (2–20 μm), 68% fine sand (20–200 μm), and 20% coarse sand (200–2000 μm). Each pot (PVC tube: height 250 cm, inner dia 83 mm) was prepared with ten 2 cm layers containing soil amounts as given by the respective bulk densities. To obtain a uniform bulk density, each of the 2 cm layers was compacted separately by application of uniaxial pressure. The organic materials (amounts and selected properties are shown in Table 1) were mixed into soil for the 2–12 cm zone before compaction (see Fig. 1 and explanation below).

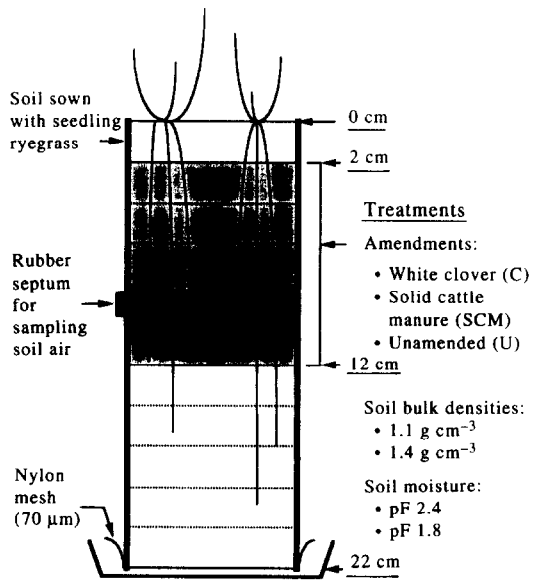

Fig. 1. Pot set-up and experimental treatments.

Table 1. Amounts and selected properties of organic materials added to soil

Organic materials	Solid cattle manure (SCM)	White clover (C)
Added:	Fresh	Dried and finely ground
Dry matter (g pot^{-1})	2.1	3.0
C concentration (%)	44.1	40.7
N concentration (%)	3.65	2.53
C:N ratio	12.1	16.1
Added N (mg pot^{-1})	75.6	75.9
^{15}N (atom %)		2.96

The soil was planted with Italian ryegrass (cv. "Tewera") to avoid elevated concentrations of mineral N, which might affect mineralization rate (Fog, 1988) and lead to gaseous N losses (Hansen *et al.*, 1993). Eleven ryegrass plants were grown for 20 days in a 2 cm soil layer that was subsequently transferred to the experimental pot. The application of actively-growing plants and the organic amendments in a relatively small soil volume was made to obtain a rapid penetration of roots through the amended soil. The function of the unamended 12–22 cm zone was to reduce plant root density and fluctuations in soil moisture. The experimental pots were placed in a growth chamber at 15°C and 18-h light (neon light tubes) with intensity of 160 μE m^{-2} s^{-1} (400–700 nm).

The water content at pF 2.4 was 0.23 mg g^{-1} dry soil and at pF 1.8, 0.31 mg g^{-1} dry soil. Pots for the pF 1.8 treatment were kept at pF 2.4 until day 31. Each 2–3 day throughout the experiment the moisture was adjusted by weight by addition of tap water at the soil surface.

Sampling

After removal from the growth chamber, the soil columns were pushed out of the pots, the shoot material was cut at the soil surface and the columns split axially into two equal halves. Roots were sampled from one of the two halves by sieving (2-mm mesh) and subsequent gentle washing in water. Shoot and root material were dried at 70°C for 2 days and ball milled.

The second of the two soil column halves was sub-divided horizontally at the borders between the layers of the planted (0–2 cm), amended (2–12 cm), and unamended soil (12–22 cm), respectively. All soil analyses were made on samples removed from the

amended 2–12 cm zone after removal of roots by sieving (2-mm mesh) and hand picking.

Soil physical analyses

Relative volume fractions of air, water, and solids were determined in five parallel 100 cm^3 soil samples (von Nitzsch, 1936) removed from a 4 cm deep soil column prepared as described above to obtain bulk densities of 1.1 and 1.4 g cm^{-3}. Pore size distributions were obtained from water retention curves determined by use of ceramic pressure plates (Richards, 1947, 1948). The pore neck diameter (d) is given by $d = 0.3/h$, where h = suction (cm water column).

The air porosity at −10 kPa matric potential (pF 2) was determined with an air pycnometer (Torstensson and Eriksson, 1936), and the total porosity was determined as the sum of air porosity and volumetric water content at −10 kPa matric potential (pF 2).

Analyses of parameters related to N turnover

Plant N and total soil N. For pots without ^{15}N-labelled clover, total N in plant material was determined by Kjeldahl analysis. For the clover treatment, plant N, total soil N and the ^{15}N abundance were determined by Isotope Services, Inc (329 Potrillo Drive, Los Alamos, NM 87544, U.S.A.) after Dumas combustion on a Carlo-Erba N/A 1500 Elemental Analyser connected to a VG Isomass Mass Spectrometer. The soil samples (stored at −18°C) were dried at 105°C overnight and ball milled before analysis.

Soil mineral N. Thirty g moist soil was shaken with 100 ml 0.5 M K_2SO_4 for 0.5 h before filtration through Whatman GF/C glass fibre filter. The extracts were stored frozen (−18°C) until determination of NH_4^+ and NO_3^- colorimetrically by flow injection analysis (Tecator, Sweden).

Microbial biomass N. The chloroform-fumigation–direct-extraction method was used to determine microbial biomass N in unamended and clover-amended soil (Brookes *et al.*, 1985). Nitrogen in the extracts was analyzed as nitrate by flow-injection analysis (Tecator, Sweden) after potassium peroxodisulfate digestion (Nydahl, 1978). Biomass N (CFE-N) was estimated using a k value of 0.69, as the soil was fumigated for 5 days (Brookes *et al.*, 1985).

Bacterial biomass N. Direct counting of soil bacteria within five volume groups was done with the Acridine Orange Direct Counting (AODC) method (Bakken, 1985). Fresh soil samples were conserved with 2.5% glutardialdehyde and stored at 4°C until counting. Biomass N was calculated from numbers and cell volume within each volume group assuming a density of 1.1 g cm^{-3}, 30% dry weight, and 12.4% N in the bacterial dry matter (Bakken, 1985).

^{15}N analysis. Determination of ^{15}N abundance in extracts from fumigated and unfumigated soil was performed by Isotope Services, Inc (329 Potrillo Drive, Los Alamos, NM 87544, U.S.A.) with an automated mass spectrometer (McInteer *et al.*, 1984). Before analysis, N in the K_2SO_4 extracts was concentrated as described by Hauck (1982). Briefly, MgO and Devarda's alloy was added to the extracts before steam distillation (Kjeltec System 1002 Distilling Unit, Tecator, Sweden), and the distillate acidulated with H_2SO_4 before evaporation to dryness. ^{15}N in plant material and soil from the 2–12 cm zone of the clover treatments was determined together with the N concentration as described above.

The ^{15}N enrichments of soil and plant N pools were calculated as atom % ^{15}N above the natural abundance measured in the unamended soil (0.373).

Selected indicators of anaerobic metabolism

Hydrogen sulphide. On day 22, the methylene blue method was used for analysis of H_2S gas in soil samples (Clesceri *et al.*, 1989, pp. 4-195–197). On day 60, occurrence of H_2S was checked in intact soil columns. Ten ml soil atmosphere was removed through the rubber sceptre in the pot wall (Fig. 1) with a syringe (Sabre, 50 × 0.8 mm needle), which thereafter was emptied against lead acetate paper (Clesceri *et al.*, 1989, p. 4-191). The detection limit for the H_2S analyses was 50 $\mu g\ S^{2-}\ l^{-1}$.

Volatile fatty acids. Sampling of volatile fatty acids were made by adding 8 g moist soil to 10-ml serum vials (Macherey-Nagel N 20-10) where it was conserved at pH < 2 with 8 ml 33% formic acid. The vials were sealed with septum-type butyl rubber stoppers and aluminium caps (Bellco) and stored (4°C) until analysis of C2–C5 acids by packed column gas chromatography (Supelco, 1990) on a Shimadzu GC-9AM with a Chromosorb W AW column and a FID. The detection limit was <1 mM.

Soil acidity. pH was measured in the 0.5 M K_2SO_4 extracts prepared for analysis of soil mineral N.

Phytotoxic compounds. Anaerobic conditions favour the production and inhibit oxidation of phytotoxic compounds (Lynch, 1985). A bioassay was carried out on day 22 [modified after Wolf (1985)]. Twenty g moist soil was shaken for 30 min with 15 ml deionized water. The water extract was filtered through a paper filter (Schleicher and Schuell 589³ Blue Ribbon), and 5 ml pipetted into each of two Petri dishes containing 50 radish (*Raphanus sativus* L. var. *radicula* cv. "Non Plus Ultra") seeds evenly distributed on a filter paper circle. Deionized water was applied as a control. The seeds were kept at 21°C, and seedlings were enumerated when 50% of the seeds in the control had germinated (24 h).

Soil atmosphere composition. Sampling of the soil atmosphere in intact soil columns was made 14, 21, 41 and 63 days after addition of organic materials. Two-way blood collection needles (Venoject 0.8 × 40 mm) with the tip slightly bent to prevent clogging, were pierced through the butyl rubber septa (Fig. 1) and connected to 3.5-ml,

evacuated, blood-collecting tubes (Venoject Plain, Silicone Coated, 65 × 10.25 mm, Code VT-030SP). Nitrous oxide, O_2 and CO_2 in the samples were analyzed on a gas chromatograph equipped with a thermal conductivity detector (TCD) and an electron capture detector (ECD). Apart from minor adjustments, the method is described in detail by Bakken *et al.* (1987). The ECD was used to determine O_2, which apparently did not harm the detector. The gas samples (0.1 ml) were injected with a syringe. The calibration gases were 1.00 and 6.43 $\mu l\ l^{-1}$ N_2O and 0.84% CO_2 standard gases (Alfax, Malmö, Sweden), atmospheric air (0.33 $\mu l\ l^{-1}$ N_2O, 0.033% CO_2, 20.9% O_2), and dilutions of atmospheric air in He to 5.24, 10.47, and 15.75% O_2.

Colony-forming bacteria. From duplicate pots sampled on day 64, 30 g fresh soil was mixed with 270 ml sterile water, homogenized in a Waring blendor for 3 × 1 min intervals with intermittent cooling in ice (3 min), and diluted further (10-fold) in Winogradsky salt solution (Pochon, 1954). Samples (0.1 ml) were then spread on agar plates.

Aerobic bacteria were enumerated after incubation of five agar plates per dilution level at 23°C for 3.5 weeks in air. A nutrient-poor (CSEA) medium described by Olsen and Bakken (1987) was used, but with tap water instead of soil extract.

Anaerobic plus facultative bacteria were counted after anaerobic incubation at 21°C for 3.5 weeks. A peptone–yeast extract–glucose medium as described by Linn and Doran (1984) was applied, and incubation carried out in a Forma Scientific Anaerobic Incubator. To determine the proportion of facultative bacteria among those growing anaerobically, 18 colonies (from each of eight clover-amended pots; low and high soil density and moisture content, respectively) were taken from the anaerobically incubated plates, plated out on the same kind of agar and reincubated in air for 1 week. Sixty-three per cent (SE = 4.4%; n = 8) of these colonies grew aerobically.

Spore-forming bacteria were enumerated after a similar anaerobic incubation of dilution plates prepared from soil stored frozen (− 18°C). The treatment deviated from the description above in that the soil was sampled from triplicate pots and pasteurized (10 min, 80°C). To evaluate whether the access of O_2 during the plating procedure affected the number of colonies, 14 additional agar plates were inoculated inside the anaerobic incubator. No effect of the O_2 availability during inoculation was detected. After the anaerobic incubation, 16 colonies were picked from each treatment, reinoculated on the same kind of agar, and incubated aerobically for 1 week. Thirty-seven per cent (SE = 5.2%; n = 8) of these colonies grew aerobically.

Statistical analysis

The results on labelled white-clover N in the various pools were tested statistically by analysis of variance (ANOVA). We used two alternative factorial models: a model for pF 2.4 included the factors BULK DENSITY (1.1, 1.4) and SAMPLING DAY (22, 42, 64, 98), and a model for day 64 included BULK DENSITY (1.1, 1.4) and SOIL MOISTURE (pF 2.4, pF 1.8). The respective residuals were used as error terms for testing of the factors BULK DENSITY and SOIL MOISTURE and the interactions BULK DENSITY × SAMPLING DAY and BULK DENSITY × SOIL MOISTURE.

For mineralization of N from added organic materials and clover-derived microbial biomass N determined by difference (amended minus unamended soil; mg pot^{-1}), a three-factor model with the factors SAMPLING DAY (22, 64) BULK DENSITY (1.1, 1.4), and AMENDMENT (C, SCM, U) was applied for pF 2.4. The effect of compaction on the difference could then be tested by the interaction BULK DENSITY × AMENDMENT. For day 64, the effects of BULK DENSITY, MOISTURE, and BULK DENSITY × MOISTURE were similarly tested by their interactions with AMENDMENT. Clover-derived bacterial biomass N on day 64 could not be tested in this model because of missing observations in unamended soil (see results). Instead, the effects of BULK DENSITY and MOISTURE on bacterial N were tested in terms of mg N kg^{-1} dry soil in a factorial model. In these models, the respective residuals were used as error terms.

The effects of SAMPLING DAY, AMENDMENT, BULK DENSITY and MOISTURE on selected indicators of anaerobic metabolism, were tested in models analogous to those applied for N mineralization determined by difference. The number of factors or factor levels was reduced depending on the pots selected for sampling. The effect of soil compaction on pore size distribution was tested in a one-way model.

RESULTS

Soil physical analyses

The major effect of soil compaction, was a reduction in the total pore volume and the volume of pores with neck dia > 30 μm (Fig. 2). The latter volume declined from 30.4% to 14.6% when the bulk density increased from 1.03 to 1.30 g cm^{-3} ($P \leq 0.001$). The smaller pore size classes increased somewhat ($P \leq 0.01$), but the effects were less pronounced. The soil matric potential, as derived from the water retention curves (not shown), was negligibly affected by soil compaction (Table 2). The measured bulk densities were lower than those intended when preparing the columns (Table 2). The reason was probably technical difficulties in removing undisturbed 100 cm^3 samples from the relatively narrow columns.

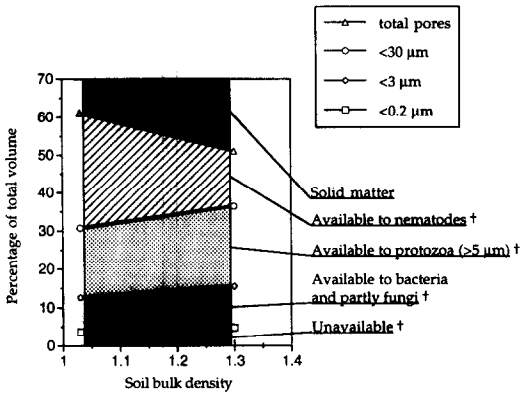

Fig. 2. Effect of soil bulk density on pore size distribution as determined from water retention curves. † van der Linden *et al.* (1989).

The fate of ^{15}N-labelled nitrogen from clover material

The effect of soil compaction and moisture on the distribution of labelled clover N is shown in Fig. 3 and Table 3. Soil compaction reduced net mineralization of labelled clover N estimated as the sum of soil mineral N and plant N ($P \leq 0.001$). This effect was more severe at the higher moisture content (Table 3), as evident from the statistically significant interaction between the factors compaction and moisture ($P \leq 0.03$). Neither compaction nor moisture affected the content of labelled soil mineral N (results not shown). The average values were 2.6, 0.6, 0.2 and 0.3 mg labelled N pot^{-1} for the days 22, 42, 64 and 98, respectively.

Complementary to the results on net mineralization, the amount of labelled organic N in the soil increased with increasing bulk density ($P \leq 0.05$). However, the higher water content at the higher bulk density did not increase the amount of soil organic ^{15}N any further (day 64).

Total recovery of ^{15}N was not negatively affected by soil compaction nor increased water content. Rather, recovery tended to be slightly higher in compacted than uncompacted soil, but the difference was not statistically significant. This suggests that the effect of compaction and increased water content in compacted soil was not due to increased losses of mineralized clover N but was caused by a real retardation of mineralization.

On days 42 and 64, additional pots with bulk densities of 1.2 and 1.3 g cm^{-3} were also included in the analyses. The results of these pots (not shown)

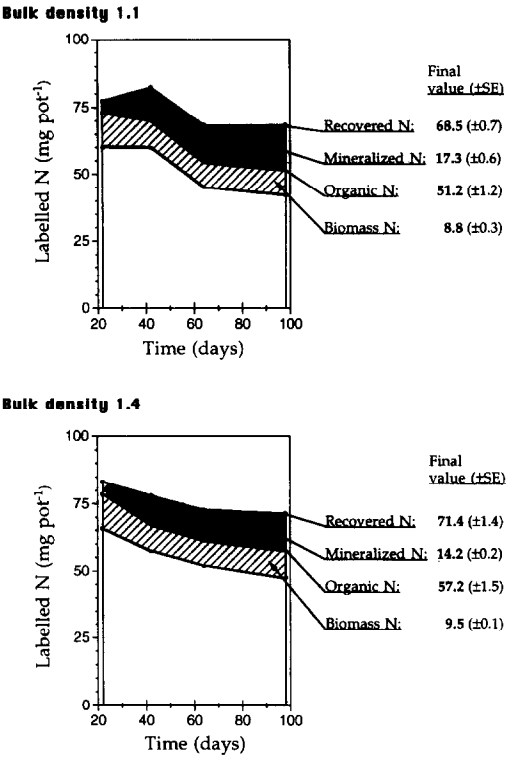

Fig. 3. Effects of soil bulk density at pF 2.4 on recovery of ^{15}N-labelled clover nitrogen in different pools (mg pot^{-1}; mean ± SE, n = 3).

were in good agreement with the pattern described above.

For the experimental period as a whole, soil compaction did not increase the amount of labelled microbial biomass N significantly. For the two last sampling days, however, there was significantly more labelled biomass at bulk density 1.4 than at 1.1 g cm^{-3} ($P \leq 0.02$). There was slightly less labelled microbial biomass at pF 1.8 than at pF 2.4 ($P \leq 0.01$).

Nitrogen mineralization and microbial biomass as determined by the difference between amended and unamended soil

Net mineralization of N (soil mineral N plus plant N; Table 4) and microbial and bacterial biomass N (Table 5) induced by organic amendments, as determined by difference, largely showed similar effects of soil compaction and moisture as those obtained by the ^{15}N technique. Nitrogen mineralization was reduced by compaction, but the difference

Table 2. Soil bulk density, pore volume, water and air content, and matric potential as determined in 100 cm^{-3} samples prepared for determination of pore size distribution

Intended bulk density (g cm^{-3})	1.10		1.40	
Measured bulk density (g cm^{-3})	1.03		1.30	
Measured pore volume (% v/v)	61.2		51.1	
Water added (mg g^{-1} dry soil)	23	31	23	31
Measured water content (% v/v)	23.7	31.9	29.9	40.3
Air content (% v/v)	37.5	29.3	21.2	10.8
Matric potential (pF)	2.4	1.9	2.3	1.8

Table 3. Effects of soil bulk density and soil moisture content (pF) on recovery of ^{15}N-labelled clover N (mg pot^{-1}) in different pools on day 64

Bulk density (g cm^{-3})	Net N mineralization (soil mineral N + plant N)		Soil organic N (total soil N − soil mineral N)		Total recovery of N (total soil N + plant N)		Microbial biomass N	
	pF 2.4	pF 1.8	pF 2.4	pF 1.8	pF 2.4	pF 1.8	pF 2.4	pF 1.8
1.1	14.2 ± 0.7*	16.5 ± 0.5	54.1 ± 1.2	53.3 ± 1.5	68.3 ± 1.7	69.8 ± 1.6	8.8 ± 0.1	8.2 ± 0.2
1.4	11.4 ± 1.3	9.5 ± 0.3	61.3 ± 1.3	62.2 ± 1.0	72.7 ± 1.7	71.7 ± 1.2	9.2 ± 0.2	8.7 ± 0.3

*Mean ± SE; n = 3.

was statistically significant on day 64 only, and only for clover pots ($P \leq 0.001$). As for the ^{15}N results, the negative effect of compaction was greater at the higher moisture content ($P \leq 0.03$).

Clover-derived microbial biomass N determined by difference was, as labelled biomass N, increased by soil compaction at pF 2.4 (Table 5). The effect was statistically significant on day 64 ($P \leq 0.02$), but not on day 22. The clover-induced microbial biomass was lower at pF 1.8 than at pF 2.4 ($P \leq 0.001$).

The effect of soil compaction and water content on bacterial biomass N (AODC-N) was only determined in clover-amended pots on day 64. Because observations from unamended soil at bulk density 1.4 and at pF 1.8 were missing, the effect of compaction and water content could only be evaluated directly in terms of mg N kg^{-1} dry soil. By this evaluation, the effect of soil compaction on bacterial biomass was confounded with the effect of lower concentration of added clover material in compacted soil. Nevertheless, at pF 2.4 AODC-N increased from 10.9 ± 0.5 (± SE; n = 3) in uncompacted to 12.6 ± 0.4 mg N kg^{-1} in compacted soil (results not shown in Figures or Tables). At pF 1.8, the corresponding values were 10.1 ± 1.5 and 11.2 ± 1.0 mg N kg^{-1}. Although not statistically significant, the effects of compaction and water content on AODC-N agreed with the results on microbial biomass N. Also, in Table 5 an indirect estimate of the effects of compaction and water content on bacterial biomass N (mg N pot^{-1}) induced by the added organic materials is presented (see Discussion).

As for labelled N, the content of soil mineral N determined by difference was unaffected by soil bulk density and moisture. For solid cattle manure, the values were 0.5, 0.1, 0.3 and 0.2 mg N pot^{-1} on the days 22, 42, 64 and 98, respectively. The corresponding values for clover-amended soil were 3.0, 0.7, 0.1 and 0.2 mg N pot^{-1} (results not shown in figures or Tables).

Soil compaction or moisture did not affect the relative amounts of ammonium and nitrate N in the soil (results not shown), which suggests that a possible reduction in aeration was not large enough to affect nitrification.

The values for unamended soil subtracted in the difference calculations are shown in Table 6. These values, which were expressed as mg N pot^{-1}, were higher in compacted than in uncompacted soil because of the larger amount of soil in pots with bulk density of 1.4 g cm^{-3}. Possible effects on turnover of native soil N were evaluated by expressing the results as mg N kg^{-1} dry soil. Doing so, no significant effect of soil compaction or water content was detected on

Table 4. Effects of soil bulk density and soil moisture content (pF) on net N mineralization from white clover and solid cattle manure (soil mineral N plus plant N; mg pot^{-1}) determined as the difference between amended and unamended soil

Sampling days Soil moisture	22 pF 2.4	42 pF 2.4	64 pF 2.4	64 pF 1.8	98 pF 2.4
Bulk density (g cm^{-3})			White clover		
1.1	1.0 ± 0.8*	10.8 ± 1.0	15.3 ± 1.5	20.1 ± 1.3	22.4 ± 1.5
1.4	−1.6 ± 0.3†	ND‡	8.4 ± 4.1	2.2 ± 1.0	ND
			Solid cattle manure		
1.1	1.0 ± 0.6	−1.3 ± 1.3	−1.4 ± 1.4	0.8 ± 1.3	6.5 ± 2.1
1.4	−1.5 ± 1.0	ND	−2.1 ± 2.2	−1.1 ± 1.9	ND

*Mean ± SE; n = 3 for both amended and unamended pots.
†n = 2 for amended pots.
‡Not determined.

Table 5. Effects of soil bulk density and soil moisture (pF) on clover-induced microbial (CFE-N) and bacterial biomass N (AODC-N) determined as the difference between amended and unamended soil (mg pot^{-1})

Sampling days Soil moisture	22 pF 2.4	42 pF 2.4	64 pF 2.4	64 pF 1.8	98 pF 2.4
Bulk density (g cm^{-3})			Microbial biomass N (CFE-N)		
1.1	17.7 ± 1.3*	12.7 ± 0.9	11.4 ± 0.6	9.6 ± 0.4	10.3 ± 0.5
1.4	18.9 ± 1.1	ND†	14.1 ± 0.6	9.6 ± 0.5	ND
			Bacterial biomass N (AODC-N)		
1.1	ND	5.1 ± 0.9	1.4 ± 0.4	0.9 ± 0.9‡	4.3 ± 1.0
1.4	ND	ND	3.1 ± 0.5‡	2.0 ± 0.8‡	ND

*Mean ± SE; n = 3.
†Not determined.
‡Subtraction term calculated from values (mg N kg^{-1}) for uncompacted soil with pF 2.4 (see Discussion).

Table 6. Values for net N mineralization, microbial biomass N, and bacterial biomass N in unamended soil that were subtracted in the difference calculations in Tables 4 and 5 (mg N pot^{-1})

Sampling days Soil moisture	22 pF 2.4	42 pF 2.4	64 pF 2.4	64 pF 1.8	98 pF 2.4
Bulk density (g cm^{-3})			Net N mineralization		
1.1	8.0 ± 0.1*	16.8 ± 0.9	20.0 ± 1.1	18.5 ± 1.2	20.9 ± 0.7
1.4	9.6 ± 0.2	ND†	23.3 ± 1.7	21.6 ± 0.8	ND
			Microbial biomass N (CFE-N)		
1.1	6.7 ± 0.6	7.2 ± 0.7	6.8 ± 0.4	7.7 ± 0.1	8.7 ± 0.4
1.4	7.5 ± 0.9	ND	7.2 ± 0.4	10.3 ± 0.5	ND
			Bacterial biomass N (AODC-N)		
1.1	ND	4.3 ± 0.1	5.1 ± 0.3	ND	5.2 ± 0.3

*Mean ± SE; n = 3.
†Not determined

N mineralization in unamended soil, although there was a slight, negative tendency for both factors (results not shown). There was no effect of compaction on microbial biomass N, but increasing the moisture from pF 2.4 to pF 1.8 increased the average value from 10.5 to 13.3 mg N kg^{-1} soil ($P \leq 0.01$; day 64).

Selected indicators of anaerobic metabolism

There were no detectable concentrations of H_2S or volatile fatty acids on any of the sampling days. However, soil compaction reduced pH (K_2SO_4; mean of sampling days) in pots added white clover and cattle manure, respectively, from 5.9 to 5.8 and 5.7 to 5.6 ($P \leq 0.01$), but not in unamended soil (pH 5.5). The increase due to amendments was statistically significant ($P \leq 0.001$). Water content had no effect on soil acidity (day 64; results not shown).

The bioassay of phytotoxic compounds carried out on day 22 showed no effect of the experimental treatments on germination of radish seeds. However, the growth of ryegrass plants in clover-amended pots was appreciably retarded until the first sampling, indicating the presence of phytotoxic compounds at an earlier stage. Plant height on day 10 was 5–6 cm in the clover pots versus 9–10 cm in the other pots (results not shown). No effect of soil compaction was visible. Plant dry matter on day 22 was also significantly lower in clover pots than in the others (Table 7; $P \leq 0.001$) despite a higher concentration of soil mineral N (4.6, 2.1 and 1.6 mg N pot^{-1} for C, SCM and U, respectively). The negative effect of clover on plant growth tended to be more severe in compacted pots ($P \leq 0.063$), again despite a higher concentration of soil mineral N (4.9 and 4.4 mg N pot^{-1} in compacted and uncompacted soil, respectively).

Table 7. Effects of soil bulk density and organic amendments on ryegrass dry matter (mg pot^{-1}) on day 22

Bulk density (g cm^{-3})	White clover manure	Solid cattle	Unamended
1.1	222 ± 33*	322 ± 27	307 ± 5
1.4	187 ± 34	335 ± 46	424 ± 5

*Mean ± SE; n = 3.

Composition of soil air (results not shown) was not affected by soil compaction nor by organic amendments at the lower moisture content. At the higher moisture content (average of samples removed on day 63), soil compaction reduced the concentration of O_2 in the soil air by 4.0 volume% ($P \leq 0.02$) and increased the concentration of CO_2 by 0.79 volume% ($P \leq 0.03$) and that of N_2O by 0.33 μl l^{-1} ($P \leq 0.02$).

There was no statistically significant effect of soil compaction or water content on colony-forming bacteria after anaerobic incubation (day 64; results not shown). Expressing the results as percentages of aerobic colony-forming units did not yield additional information. The organic amendments, particularly clover, significantly increased the number of colony-forming bacteria (Table 8).

DISCUSSION

Soil compaction reduced N mineralization from the added organic materials and increased labelled N retained in microbial biomass and soil organic matter. Van der Linden *et al.* (1989) found similar effects on the mineralization of C after addition of ^{14}C-labelled glucose or bacteria to a silt loam. However, in their experiment the reduced mineralization was not reflected as a larger incorporation of label in the soil biomass. Contrary to these observations, Kaiser *et al.* (1991) in a laboratory experiment found that soil compaction increased evolution of ^{14}C-CO_2 from added straw, and in a field trial they observed that compaction reduced microbial biomass and total organic C. The authors attributed their results to reduced microbial efficiency of C assimilation because of O_2 deficiency in compacted soil.

There are two possible explanations to our findings. Compaction may have led to more anaerobic microsites, resulting in inhibited degradation of lignified clover material, i.e. inhibited gross mineralization of labelled N (Gale and Gilmour, 1988). Oxygen deficiency would also increase the gaseous loss of mineralized N (Hansen *et al.*, 1993). However, we did not find any clear evidence of anaerobic metabolism in compacted soil (see below) nor did van der Linden *et al.* (1989) in

Table 8. Effects of organic amendments on aerobic, anaerobic plus facultative, and spore-forming anaerobic plus facultative bacteria (colony-forming units g^{-1} dry soil) on day 64 in soil with pF 1.8 (average of the two bulk densities)

	White clover	Cattle manure	Unamended	SE (pooled)	$P \leq$
Aerobic	3.9×10^8	2.4×10^8	0.6×10^8	0.3×10^8	0.001
Anaerobic + facultative	8.9×10^6	2.1×10^6	1.8×10^6	1.2×10^6	0.01
Spore-forming an. + fac.	9.7×10^5	9.0×10^5	6.7×10^5	0.8×10^5	0.053

their experiment. Also, our results on total ^{15}N recovery showed that gaseous loss of N was not larger in compacted than in uncompacted soil.

The other explanation is that compaction may have increased the soil's capacity to protect microbial biomass and metabolites against further degradation. It may be assumed that bacteria and fungi live in pores with diameters > 0.2 μm, protozoa > 5 μm, and nematodes > 30 μm (van der Linden *et al.*, 1989). In our experiment, soil compaction considerably reduced the volume of pores available to nematodes, organisms that substantially increase the turnover of N by their grazing on microorganisms (Elliott *et al.*, 1980; Woods *et al.*, 1982; Griffiths, 1986). In addition, the volume of pores unavailable to cellular organisms and pores available only to bacteria and fungi increased slightly (Fig. 2). The higher microbial and bacterial biomass N measured in compacted soil supports the assumption that physical protection was the underlying mechanism.

At the end of the experiment, the increase in labelled biomass N accounted for 22% of the reduction in clover N mineralization and 11% of the increased retention of labelled organic N. The discrepancy between the increase in biomass N on the one hand and the effect on N mineralization and organic N retention on the other hand does not invalidate the explanation of physical protection. A substantial part of N immobilized by active microorganisms is continuously transformed to non-biomass substances (Breland and Bakken, 1991). The further turnover of these substances may also be retarded by soil compaction: reduced grazing may reduce microbial activity (Hunt *et al.*, 1977; Woods *et al.*, 1982; Griffiths, 1986), and organic matter may be enclosed in pores physically inaccessible to microorganisms (Elliott and Coleman, 1988).

Unfortunately, microscopic counts of bacteria were not determined for compacted, unamended soil nor at pF 1.8. Therefore, the effect of compaction and water content could only be evaluated directly in terms of mg N kg^{-1} dry soil. However, bacterial biomass N induced by organic amendments with these treatments was indirectly estimated by subtracting values calculated from AODC-N (mg kg^{-1}) in uncompacted soil at pF 2.4. This seemed justified since compaction had no effect on CFE-N (mg kg^{-1}) in unamended soil.

The negative effect of soil compaction on net N mineralization was larger at the higher moisture content, but this was not proportionally reflected in the retention of labelled organic N. The observed result was hardly caused by increased gaseous losses of N since compaction did not reduce the total recovery of labelled N, and the N_2O concentration of the soil air increased only slightly.

Among the selected indicators of anaerobic metabolism, a slightly reduced pH in compacted soil and a transient inhibition of initial plant growth were the only signs of more reducing conditions due to compaction at the lower moisture content. Thus, anaerobiosis is an unlikely explanation for the retarded N mineralization in this treatment. Even at the higher moisture content, the reductions observed in O_2 concentration were too small to induce a predominantly anaerobic metabolism, although microsites may have been anaerobic.

In conclusion, soil compaction reduced mineralization of labelled clover N by 18% as compared to mineralization in uncompacted soil, while retention of labelled N in soil organic matter and microbial biomass increased. Total ^{15}N recovery did not decrease and no evidence of anaerobic conditions was found in compacted soil. Consequently, the reason for reduced mineralization could not be increased gaseous losses of mineralized N nor retarded decomposition of clover residues due to O_2 deficiency. The volume of pores available to nematodes, however, was substantially reduced by compaction. Therefore, we hypothesize that the mechanism by which soil compaction reduced N mineralization was retarded turnover of organic materials and microbial biomass owing to increased physical protection.

Acknowledgements—This work was funded by the Research Council of Norway and the Borgestad II Legacy (^{15}N analyses). We are thankful to MSc J. F. Hanssen for advice regarding anaerobic decomposition products, Dr L. E. Haugen for conducting the pore size analysis, and Dr L. R. Bakken for useful discussions during experimentation and writing.

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