NITROGEN IN SOIL *NOSTOC* MATS: FORMS, RELEASE AND IMPLICATIONS FOR NUTRIENT CYCLING IN ANTARCTICA

LAURENCE G. GREENFIELD

Department of Plant and Microbial Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

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ABSTRACT

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Cyanobacterial mats dominated by *Nostoc commune* were collected from wet soils in Antarctica, the Arctic and New Zealand, analysed for forms of nitrogen (N) and subjected to decomposition as shown by N mobilisation and weight loss due to microbial metabolism. Mats incubated at low moisture levels (4%) did not decompose significantly. Ground mats decomposed at low temperatures ± freezing events with up to 20% of total mat N becoming mobilised and 15% of initial weight being lost as metabolic CO_2 and water after 30 d. Based on dry weight and N content (m^{-2} basis) of biota in various parts of the world it is suggested that *Nostoc* mats in the Garwood Valley, Antarctica contain a high proportion of dormant and dead cells. The physical nature of *Nostoc* mats may be more important than the effects of low temperature on the microbial decomposition and nutrient release from dead *Nostoc* cells.

KEYWORDS: Cyanobacterial mats - Antarctic soils - decomposition - nitrogen.

INTRODUCTION

Microbial mats occur on wet ahumic mineral soils at high latitudes in polar regions. These mats are often dominated by cyanobacteria especially *Nostoc* species and particularly *N. commune* (Vincent *et al.* 1993, Cameron *et al.* 1978). These mucilagenous mats can cover and stabilise large areas of polar soils and, from visual observations, would be expected to comprise a large dry weight on a unit area basis. High biomasses are indirectly suggested by chlorophyll-a analyses of microbial mats (Vincent *et al.* 1993). *Nostoc* mats constitute potentially large nutrient reservoirs and, on the death of *Nostoc* cells, decomposition would release these nutrients including nitrogen (N) back into the terrestrial ecosystem. Taxonomic and physiological work has been reported on *Nostoc* mats, (Vincent *et al.* 1993) but no quantitative decomposition work has been reported, (Stahl 1995, and pers comm.)

This paper reports the forms of N in *Nostoc* mats and the effects of temperature and moisture on the extent of mat decomposition.

MATERIALS AND METHODS

Microbial mats on upland and lowland wet soils were collected in Antarctica (Jan. 1993), the Arctic (Aug. 1995) and New Zealand (Dec. 1995) during the growing season. Mats, dominated by *Nostoc commune*, were immediately air dried or kept frozen until it was convenient to air dry. In Jan. 1996 the dry samples were ground (<1 mm) before ash (550°C, 4h), pH (2:1w:v) and total N (semi micro Kjeldahl Bremner 1965) determinations. Due to logistics, only small amounts of mats were collected in the Arctic (Chukchi Peninsula 66°N 174°W, Point Barrow 71°N 156°W, Devon Island 75°N 86°W) and equal amounts of these samples were pooled. In Antarctica, samples were obtained from an extensive *Nostoc* mat in the

Garwood Valley (78°S and 164°E) and in New Zealand, *Nostoc* samples were collected from growths in glacier and moraine pools and wet soils on or adjacent to the Franz Joseph Glacier region (43°S 170°E).

Forms of N in *Nostoc* samples (duplicates) were determined by 6 M HCl reflux hydrolysis, followed by neutralisation and fractionation of hydrolysates according to Greenfield (1979).

Decomposition was estimated by weight loss and N mobilisation. Weight loss was determined by mixing ground samples, (about 0.5 g ash free basis) with 5.0g ignited Garwood Valley sandy soil in preweighed 50 ml glass tubes. Moisture contents of the mixtures were adjusted to 4 or 25%, on a mixture dry weight basis, by addition of distilled water. Triplicate tubes were capped with polythene and incubated with no inoculation for up to 30 days in the dark under various temperature regimes. One set of samples was subjected to a constant 4°C, whilst another set was similarly incubated but frozen for 3 hours each day (variable 4°C). Some Antarctic samples were incubated at a constant 10°C, whilst another set was incubated at 20°C but frozen for 3hr each day (variable 20°C) at 25% moisture levels and one sample set was incubated at a constant 20°C but with a 4% moisture level. Some Antarctic samples were treated with $CHCl_3$ to kill cells before incubation in order to distinguish the effects, if any, of a decomposer microbial population on samples which, although air dried, would be expected to contain a proportion of viable cells. These samples were placed in a desiccator containing $CHCl_3$ and fumigated for 48 hr by evacuating the desiccator until the $CHCl_3$ boiled. After 48 h, $CHCl_3$ was removed by repeated evacuation with a high pressure pump. These samples were reinoculated with Antarctic microbes by adding 0.1 ml of a suspension made by shaking 0.1 g air dried Antarctic *Nostoc* mat with 10 ml sterile distilled water. Intact cores (5 cm diameter) taken from Antarctic mats were also examined for decomposition (weight loss and N mobilisation) but these were placed on the surface, not mixed in, with sand in incubation vessels.

Following incubation, tubes were dried for 24 hours at 105°C, cooled in a desiccator and reweighed to determine weight loss due to microbial action. Controls consisted of similarly prepared tubes but each contained 0.5 ml $CHCl_3$ to prevent microbial growth. These tubes were sealed with rubber bungs. After incubation, the weight losses of these controls (mostly moisture present in initial air dry samples) were subtracted from losses recorded for experimental tubes.

N mobilisation was conducted using similar amounts of materials and temperature and moisture conditions to those above but 100 ml flasks were used, containing vials with 1 ml of 1 M H_2SO_4 suspended over the mixtures to trap any ammonia evolved. Following incubation, mineral-N was extracted with 2 M KCl and estimated by titration following steam distillation with MgO and Devardas alloy and collection of ammonium in boric acid. Mineral-N at the start of incubation was determined for each sample and this value subtracted from the appropriate experimental flasks.

Mean results from triplicate analyses (± SD) are reported on an initial dry weight (ash free) or initial N basis.

RESULTS

On an ash free basis, Arctic, Antarctic and New Zealand *Nostoc* mat samples contained similar amounts of N (mean = 3.7% N) and not more than 1% of sample N was mineral N at day 0. The pH of aqueous extracts from these samples was between 7.0 and 7.4. Samples, regardless of origins showed similar N distributions (Table 1). Excepting intact cores, *Nostoc* mats collected from the three distinct regions were decomposed to a similar extent with a mean weight loss of 15% (range 13-19) and a mean N mobilisation value of 19% (range 16-23) occurring at a constant or variable 4°C (Table 2). Decomposition did not occur with samples incubated at 4% moisture level irrespective of incubation temperature. At 25% moisture

Table 1. N distribution analyses of *Nostoc* mats from three geographical regions. Mean results expressed as a % of total sample N (± SD). [a] ash free basis, [b] HUN = hydrolysable unidentified N, [c] insoluble N = N insoluble in 6 M HCl.

Sample origin	%N[a]	NH_4-N	Hexosamine-N	∝ amino-N	HUN[b]	insoluble N[c]
Arctic	3.8 (±0.1)	11 (1)(±1)	3 (±1)	53 (±1)	26 (±1)	7 (±1)
Antarctica	3.4 (±0.1)	9 (1)(±1)	3 (±1)	52 (±2)	28 (±2)	8 (±1)
New Zealand	3.9 (±0.1)	9 (1)(±1)	2 (±1)	56 (±1)	27 (±1)	6 (±1)

level, more decomposition occurred at 10°C (means = 23% wt loss and 23% N mobilisation) and 20°C (means = 26% wt loss and 30% N mobilisation) than that which occurred at 4°C. Intact cores from Antarctic *Nostoc* mats underwent less decomposition than similar but ground samples (Table 2), whilst fumigated, but inoculated, ground samples showed similar levels of decomposition to corresponding but unfumigated samples (Table 2).

DISCUSSION

All the samples used in this work, although collected in summer or early fall, probably contained variable proportions of live and dead cells. Live mat cells would include unknown amounts of active and inactive cells. Such a criticism can be levelled at most work on *Nostoc* mats so far reported, (Vincent *et al.* 1993, Stahl 1995). In addition, with the exception of intact-cores, samples were ground to reduce mineral soil contamination problems and some incubations were performed at constant temperature and moisture levels, unrealistic to some extent, of the prevailing field conditions from where samples originated. The imposition of a daily freeze event and a temperature of 4°C approaches conditions found on wet soil surfaces in the Antarctic summer whilst similar freezing combined with 10°C approaches field conditions found in some Arctic and montane New Zealand soils in summer (Brown & Veum 1974, Greenfield pers. obs.). The comminution of organic matter leads to an increase in surface area and substrate accessibility and can be ac complished by animals and physico-chemical weathering (Swift *et al.* 1979). In polar regions, soil fauna are sparse and physico-chemical factors although the main weathering agents are probably quite slow (Campbell & Claridge 1987). Mesofauna live within microbial mats (Suren 1990) and

Table 2. Weight loss (% initial dry wt.) and N mobilisation (% initial N) from *Nostoc* mats collected from different geographical areas incubated for up to 30 d. Data as means of three replicates ± SD. [a] Does not include time frozen, [b] intact cores used, [c] fumigated sample.

Sample Origin	Temperature °C	Moisture %	weight loss %	N mobilisation %	incubation time d.
Arctic	4	25	16±2	21±2	30
Arctic	Variable 4	25	14±3	23±2	26[a]
Arctic	4	4	<1	<1	30
Antarctic	4	25	13±2	19±2	30
Antarctic [b]	4	25	4±2	7±3	30
Antarctic [c]	4	25	16±2	17±3	30
Antarctic	Variable 4	25	15±1	18±2	26
Antarctic	4	4	<1	<1	30
Antarctic	10	25	23±3	23±2	30
Antarctic	Variable 20	25	26±3	30±2	26
Antarctic	20	4	<1	<1	30
New Zealand	4	25	17±3	18±2	30
New Zealand	Variable 4	25	19±3	16±3	26
New Zealand	4	4	<1	<1	30

probably have a grazing role but the extent and importance of these organisms in nutrient cycling does not appear to be large (Vincent *et al.* 1993). When compared to ground samples, fumigated or otherwise, intact cells in Antarctic *Nostoc* mats underwent considerably less decomposition and this suggests that, potentially, decomposition can be extensive at low temperature (Table 2) provided adequate moisture and a sufficiently large amount of substrate is exposed. Guthrie and Bourke (1981) observed 100% hydrolysis of urea by microbes in soil incubated at 2°C for 21d. Reichardt and Dieckmann (1985) reported cellulases with temperature optima of 0°C produced by bacteria decomposing organic matter, probably cell wall, from *Hismantothallus grandifolius* (a brown alga) common in sea water around the South Shetland Islands in maritime Antarctica. Davis (1986) reported weight losses of up to 25% y^{-1} for mosses decomposing in peat soils in the maritime Antarctic. This, and earlier points, suggest that enzymes from cold tolerant organisms can function at low temperatures provided that their substrates are accessible to the enzymes. This may not always be the case. The results in Table 2 for intact cells may support the suggestion that the physical nature or structure of mats may be a factor limiting decomposition. *Nostoc* mats are of a mucilagenous and leathery texture with each densely packed filament surrounded by extracellular polysaccharides. Such an arrangement may, in the absence of grazing invertebrates, lead to anaerobic conditions in the lower regions of microbial mats (Fallon & Brock 1979). Cell wall structure would also invoke chemical structures and Gunnison and Alexander (1975 a, b) have suggested that particular cell wall components in some algae were responsible for either the susceptibility or resistance to enzymes shown by these organisms. Stahl (1995) suggested that little is known about the decomposition of cyanobacterial mucilage although algal mucilage has been found to be quite susceptible to bacterial enzymes (Coveny & Wetzel 1989).

Chemical inhibition of decomposition can probably be discounted in the present work since more decomposition occurred in ground than intact samples (Table 2) and water soluble extracts from cyanobacterial mats have been found to mobilise N at 4°C (Greenfield 1989).

Most of the N in the NH_4-N fraction (Table 1), is derived from amide-N and most of the hydrolysable unidentified N (HUN) fraction is derived from amino-N which is not in the α form (Greenfield 1972). These, together with the α amino-N values in Table 1 suggest that 70-80% of the N in the *Nostoc* mats was initially present as protein. Proteins isolated from algal and microbial cells are not particularly resistant to decomposition (Verma *et al.* 1975). However, less decomposition would be expected to occur if these proteins were physically protected by cell walls or chemically complexed with non-nitrogenous compounds (Kassim *et al.* 1981, Swift *et al.* 1979).

Vincent and Howard-Williams (1989) reported a small decrease in chlorophyll-a content of intact *Nostoc* mats incubated in the dark at 5°C for 31 d. and suggested that large quantities of chlorophyll may persist undecomposed in *Nostoc* mats due to low temperature. Their work could also support the suggestion that the physical nature of microbial mats is important in reducing microbial decomposition at low temperatures. This physical protection would seem to be largely destroyed or reduced with increasing temperature for Vincent and Howard-Williams (1989) observed extensive (>99%) decomposition of chlorophyll and phaeophytin in *Nostoc* cores incubated at 25°C for 31 d. Such a temperature may have encouraged the proliferation of metabolically versatile mesophilic microbes whose growth had been checked at low temperatures. Fallon and Brock (1979) noticed that during cyanobacterial and algal decomposition at 25°C, chlorophyll and protein contents de-

creased with a concomitant rise in the appearance of mineral N and phosphorus. Interestingly, chlorophyll degradation ceased and protein decomposition was much reduced when these samples were incubated under anaerobic conditions. Shilo (1970) reported a bacterium which could decompose 8 cyanobacterial species including *Nostoc* and *Oscillatoria* species.

Nostoc mats examined in the Garwood Valley, Antarctica had a mean dry weight of 215 g m^{-2} (ash free basis) and contained 7.3 g organic N (Greenfield & MacLean 1992). This dry weight approaches and often exceeds the dry weights, for example, of mosses, roots and microbial biomass in many heavily vegetated temperate ecosystems (Persson 1980, Persson *et al.* 1980, Van Tooren 1988). The N content of 7.3g often exceeds that contained in the plant standing crop (m^{-2} basis) at vegetated sites in a latitudinal gradient from 75°N to 40°N (Van Cleve & Alexander 1981). All of the soils at these sites together with those in many low latitude Antarctic sites (eg. Signy Island (60°S) contained 200-1700 g m^{-2}), had amounts of N in the form of dead organic matter which is potentially available to supply the growing needs of biota at these sites. With the exception of geothermal (Broady *et al.* 1987, Bargagli *et al.* 1996) and guano (Speir & Cowling 1983) soils, continental Antarctic soils do not contain organic matter and are not usually subjected to leaching (Campbell & Claridge 1987). If the Garwood soil *Nostoc* mats consisted entirely of active biomass it would be difficult to envisage how they could obtain sufficient nutrients to sustain such a high level of biomass despite their autotrophic attributes. Biological N fixation by these Garwood soil *Nostoc* mats have been estimated on two field trips (Broady *et al.* 1987, Greenfield & MacLean 1992) to be no more than 1g N m^{-2} y^{-1}. Most of the N mobilised during decomposition (Table 2) during the growing season in the Garwood Valley (Dec-Jan) would be flushed into the adjacent Garwood river by the main source of melt water to this soil system - an unnamed hanging ice cap. Nutrient levels and flows have not been determined in this system, but some Antarctic stream waters contain high concentrations of inorganic N and P (Vincent *et al.* 1993).

Soil *Nostoc* mats in continental Antarctica are likely to grow slowly and contain an unknown but possibly high proportion of dead cell that provide them with one source of N. Cell decomposition is likely to be slow in large part because of the physical structure of *Nostoc* mats rather than because low temperature inhibits decomposition. For a fuller understanding of the factors and processes involved in the establishment, proliferation and decomposition of soil cyanobacterial mats, more work is clearly needed.

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