Fertilizer Research **33**: 47–57, 1992.

Nitrogen fertilizer value of activated sewage derived protein: effect of environment and nitrification inhibitor on NO_3^- release, soil microbial activity and yield of summer cabbage

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Received 31 July 1991; accepted in revised form 11 June 1992

Key words: Ammonium, cabbage, nitrate, nitrogen fertilizer, nitrification inhibitor, organic fertilizer, soil microbial activity, temperature

Abstract

Yield response of summer cabbage (*Brassica oleracea* var *capitata* cv. Hispi F_1) to N applied as organic (activated sewage sludge derived protein [Protox] and dried blood) and inorganic (ammonium nitrate, ammonium sulphate, sodium nitrate and urea) fertilizers was compared in relation to the N availability characteristics of the materials. Effects of the nitrification inhibitor dicyandiamide (DCD) on N release, crop yield and N status were also assessed. In addition CO_2 efflux was measured from amended soil to determine effects of fertilizer application on soil microbial activity. The organic N sources were mineralized quickly on application to soil and exhibited similar patterns of NH_4–N depletion and NO_3–N accumulation as functions of thermal-time as with mineral fertilizers. However, the yield response to organic N was marginally smaller (though not significantly) compared with mineral forms; probably because less N was released to the crop. This was reflected in smaller total N concentrations and N recoveries in plants supplied with organic fertilizer. Applied DCD increased the thermal-time for complete nitrification of NH_4–N sources and raised the total N content of the crop, but had no overall effect on crop growth. In contrast to inorganic N sources which generally reduced CO_2 efflux from soil, application of protein-based fertilizers increased the rate of soil microbial activity directly by raising substrate availability. Sewage sludge derived protein provided an effective alternative to mineral fertilizers for the nutrition of summer cabbage whilst minimising stress of the soil environment which may occur following the application of conventional forms of inorganic N to the soil.

Introduction

There is increasing interest in the potential benefits of recycling organic waste materials, such as sewage sludge, as fertilizers and soil conditioners for crop production. In addition, there is now increased emphasis on processing sewage sludges to produce novel dry granular, pelletized and fortified organic-based materials which potentially have more specialized uses in agriculture and horticulture compared with conventional agricultural disposal [30]. However, there are few data available on the effects of these new materials on crop growth and quality, and on soil properties, compared with conventional mineral fertilizers.

Activated sewage sludge contains a large proportion of protein-N and, in a processed form, has potential as a concentrated organic N fertilizer. For example, Protox (Protox Products Ltd)

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is produced from surplus activated sludge by inducing flocculation through pH manipulation, which also reduces the heavy metal content of the sludge. The flocculated material is dried by heating, rendering it sterile and safe to handle, and is subsequently processed into granules. Activated sewage sludge derived protein has already shown potential as a N fertilizer for vegetable crops [24]. However, more information is required on the N release characteristics and crop responses to this material under field conditions for the efficient utilization of the available nutrients supplied, compared with other organic and inorganic N sources.

The benefits of using organic N fertilizers in preference to mineral forms are thought to arise from the slower release of N which more closely matches crop requirements [29]. Nitrogen losses by, for example, leaching and denitrification may therefore be smaller compared with mineral forms. Recently, however, soil organic matter and organic manures have been implicated as the major sources of NO_3–N leached from arable soils contaminating potable groundwater supplies [16]. Nitrification inhibitors may potentially reduce NO_3^- losses by leaching from NH_4–N liberating fertilizer materials, including organic N sources, by maintaining N as NH_4^+ which is less susceptible to loss from the soil by this route [22]. Nitrification inhibitors may also improve the efficiency of N fertilizer use by increasing the N recovery and yield response of crop plants compared with fertilizer applied alone.

Fertility of soils used for crop production depends on soil organic matter content and soil microbial activity. However, there is much conflicting evidence concerning the effects of inorganic fertilizers on soil microbial activity. In particular, decreasing soil water potential following mineral N application, and declining pH resulting from nitrification of NH_4^+ sources are known to reduce the activity of soil micro-organisms [4, 27]. In contrast, inorganic N fertilizers can raise microbial activity indirectly by increasing substrate availability through improved crop growth [6]. Effects of protein-based N fertilizers on soil microbial activity under field conditions have not been investigated previously.

The purpose of this study was to assess N transformations in soil amended with sewage derived protein and other organic and inorganic N fertilizers, supplied with or without a nitrification inhibitor under contrasting environmental conditions in the field. Effects on the growth and N content of summer cabbage are described. In addition, CO_2 release into amended soils was compared to assess affects on soil microbial activity. The suitability of activated sewage sludge derived protein as a potential substitute for applied inorganic N fertilizers is discussed.

Materials and methods

Environmental conditions and fertilizer treatments

Summer cabbage (*Brassica oleracea* var. *capitata* cv. Hispi F_1) were grown on a sandy loam soil in a polyethylene covered tunnel (5.6 m by 19.1 m) and on an adjacent unprotected site to achieve two contrasting soil temperature and soil moisture content regimes. The mean soil temperature in the protected and unprotected environments was 20.6 and 16.6°C respectively over the experimental period. The soil moisture content in the protected site was generally lower at 11.7% compared with 15% in the unprotected site. However, irrigation applied to the protected site at the beginning of the experiment, and also after 20 days, increased soil moisture content to 17% which subsequently declined to the base level in the following 10 days after application. Nitrogen was applied as Protox (10% N), dried blood (13% N), ammonium nitrate (34.5% N), ammonium sulphate (21% N), sodium nitrate (16% N) and urea (46% N) at a single rate equivalent to 250 kg N ha^{-1}. The fertilizers were applied to small plots (1.44 m^2) and incorporated into the soil by hand to a depth of 70 mm. Urea was omitted from the protected site and control plots received no N fertilizer. The N materials were supplied with and without nitrification inhibitor dicyandiamide (DCD) (66% N) applied at a rate equivalent to 28 kg N ha^{-1} corresponding to 1/9 of the fertilizer N [3]. Phosphorus and K were supplied as triple superphosphate (20% P) and potassium chloride (50% K) at rates equivalent to 154 kg P ha^{-1} and 249 kg K ha^{-1} according to recommended practice [18]. The

treatments were arranged as a split-plot for DCD application with three randomized blocks at each site.

Cultural techniques

Seeds of summer cabbage (*Brassica oleracea* var. *capitata* cv. Hispi F_1) were sown on 11 April 1985 in standard seed trays (0.22 m × 0.34 m) containing 'Levington Universal Compost'. Seedlings were selected for uniformity and transferred to peat blocks (30 mm wide by 50 mm deep) of 'Levington Blocking Compost' after 6 days. Experimental plants were set out at a spacing of 0.3 m × 0.3 m on 4 June 1985. Plots were separated by a single row of guard plants. Cabbages were harvested 7 weeks after planting out in the field. Plant material was dried in a forced-air oven set at 85°C for 48 h and stored for chemical analysis.

Soil microbial activity

Soil microbial activity and N transformations in soils amended with the N fertilizers were studied in small plots (0.9 m^2) arranged in three randomized blocks, adjacent to the planted area, at both sites. Nitrogen, P and K fertilizers and DCD were incorporated at rates equivalent to those supplied to the planted area.

Soil microbial activity was determined by measuring CO_2 evolution from soil using an infrared gas analyser (IRGA) (Grubb Parsons, SB2). The gas analysis system for measuring CO_2 concentration in soil air was modified from that previously described by Smith and Hadley (1990) [26]. Briefly, air was continuously drawn from field soil through 13 air lines (PVC, 7.5 mm internal diameter) by an air pump (Charles Austen, M361) connected via a manifold and solenoid switching system to the IRGA. Gas samples from soil plots were drawn through inverted plastic funnels (100 mm diameter) buried to depth of 30–40 mm. The IRGA was calibrated to measure absolute CO_2 concentration (range, 300–2000 ppm). Each channel was sampled sequentially for 10 minutes every 2 h. The IRGA was fitted with interference filters to suppress sensitivity to water vapour, and regularly calibrated using gas-mixing pumps (Wostoff, ISA27/2 and ISA27/3). The IRGA output was recorded on a potentiometric chart recorder (Smiths Industries, Servoscribe). Soil temperature was monitored at a depth of 40 mm in both environments with copper-constantan thermocouples linked to a multi-point temperature recorder (Honeywell Brown, Type 15).

Carbon dioxide concentration in soil air from half of the plots was monitored for periods of 2 to 4 days. Sample lines were then systematically moved through the plots, such that each fertilizer combination was monitored on alternate sampling periods.

Nitrogen content of soils and plants

Soil samples were taken from the bare-ground plots at 4–5 day intervals with a soil corer (45 mm diameter) to a depth of 70 mm. Soil samples were bulked for each treatment and thoroughly mixed. The samples were then divided and half were air-dried and stored at 4°C while the remainder were stored fresh at −20°C for chemical analysis.

Oven-dried plant material was ground with a hammer mill (Tecator, Cyclotec 1092 Sample Mill) before analysis. Air-dried soil samples were ground to pass through a 2 mm sieve and fresh soils were passed through a 5.6 mm sieve. Moisture content of soil samples was determined by drying in a forced-air oven at 85°C for 24 h. Results of chemical analyses are expressed on a dry weight basis.

Fresh soil samples were extracted with 2M KCl for NO_3–N and NH_4–N determination with a Heli-flow Flow Injection Analyzer (WPA, Cambridge). Nitrate-nitrogen was extracted from plant material with deionized water and measured with a Heli-flow analyzer [23]. The pH of air-dried soil samples was measured by standard methods [17]. Total N concentration in plant material was determined using a block digester [12] and a Chemlab System 4 autoanalyzer [7]. Nitrogen recovery was estimated by the difference method [2].

Statistical treatment of data

Analysis of variance was carried out on the cabbage yield and N content data. The environ-

ments were not replicated so that statistical comparisons were only made between treatments at each site. Broken-stick functions describing relationships between N transformations in soil and thermal-time were determined by linear regression analysis.

Results and discussion

Crop yield

Nitrogen fertilizer application significantly increased cabbage yield compared with the control, irrespective of the form supplied (Table 1). However, crop yield was marginally smaller with the protein-based materials than with inorganic sources. The dry matter content of cabbage was similar for all the applied fertilizers at 7.2% across both environments, but was smaller compared with the control which had a dry matter content of 9.0%.

Other studies [29] have indicated yield responses of vegetable crops to organic N are smaller than with equivalent rates of mineral N, particularly for crops with large N requirements. However, similar yield responses to organic and inorganic N have also been reported [15]. These conflicting reports probably arise because factors which affect yield responses to organic N sources vary between studies. Such factors include the form of organic material supplied, environmental conditions, rates of N mineralization, crop type and N requirements, and soil type. However, incubation studies [26], indicate the main reason why crop responses to protein-based N fertilizers may be smaller is because only a proportion of their N content is released to the crop. The remaining N is highly recalcitrant, the slow release of which is likely to benefit crops in the long-term.

Soil N status

For each fertilizer type, concentrations of NH_4–N and NO_3–N in soil from both protected and unprotected sites varied as similar functions of accumulated soil temperature (above 0°C) (Figs. 1 and 2). In general, there were two contrasting patterns of changes in soil NH_4–N and NO_3–N concentration depending on the form of applied N. Ammonium-N concentration in soil amended with Protox, ammonium nitrate, ammonium sulphate and urea declined from initial high levels due to nitrification, which led to a concomitant increase in the level of NO_3–N. Dried blood differed from these fertilizers because NH_4–N concentration increased to a maximum first, due to mineralisation of the organic N, before subsequently declining. The NH_4–N concentration of soil supplied with Protox was initially larger compared with dried blood because the sludge-based material contained soluble NH_4–N derived from the production process. Both organic fertilizers were degraded quickly and did not exhibit slow-release characteristics such that the overall pattern of NH_4–N depletion and NO_3–N accumulation in soil was similar to that which occurred with inorganic NH_4^+ sources. The second pattern of change in soil NH_4–N concentrations occurred in soil amended with sodium nitrate and in control plots. Here, NH_4–N was

Table 1. Effect of N source and nitrification inhibitor (DCD) on the yield response of summer cabbage (kg plant^{-1} fresh weight)

	Fresh weight (kg plant^{-1})			
	Unprotected site		Protected site	
N source	Without DCD	With DCD	Without DCD	With DCD
Protox	0.48	0.45	0.40	0.45
Dried Blood	0.48	0.45	0.42	0.49
Ammonium nitrate	0.48	0.51	0.41	0.48
Ammonium sulphate	0.47	0.51	0.45	0.50
Urea	0.48	0.51	–	–
Sodium nitrate	0.47	0.55	0.44	0.43
Control	0.29	0.30	0.25	0.28
SED	0.051		0.096	

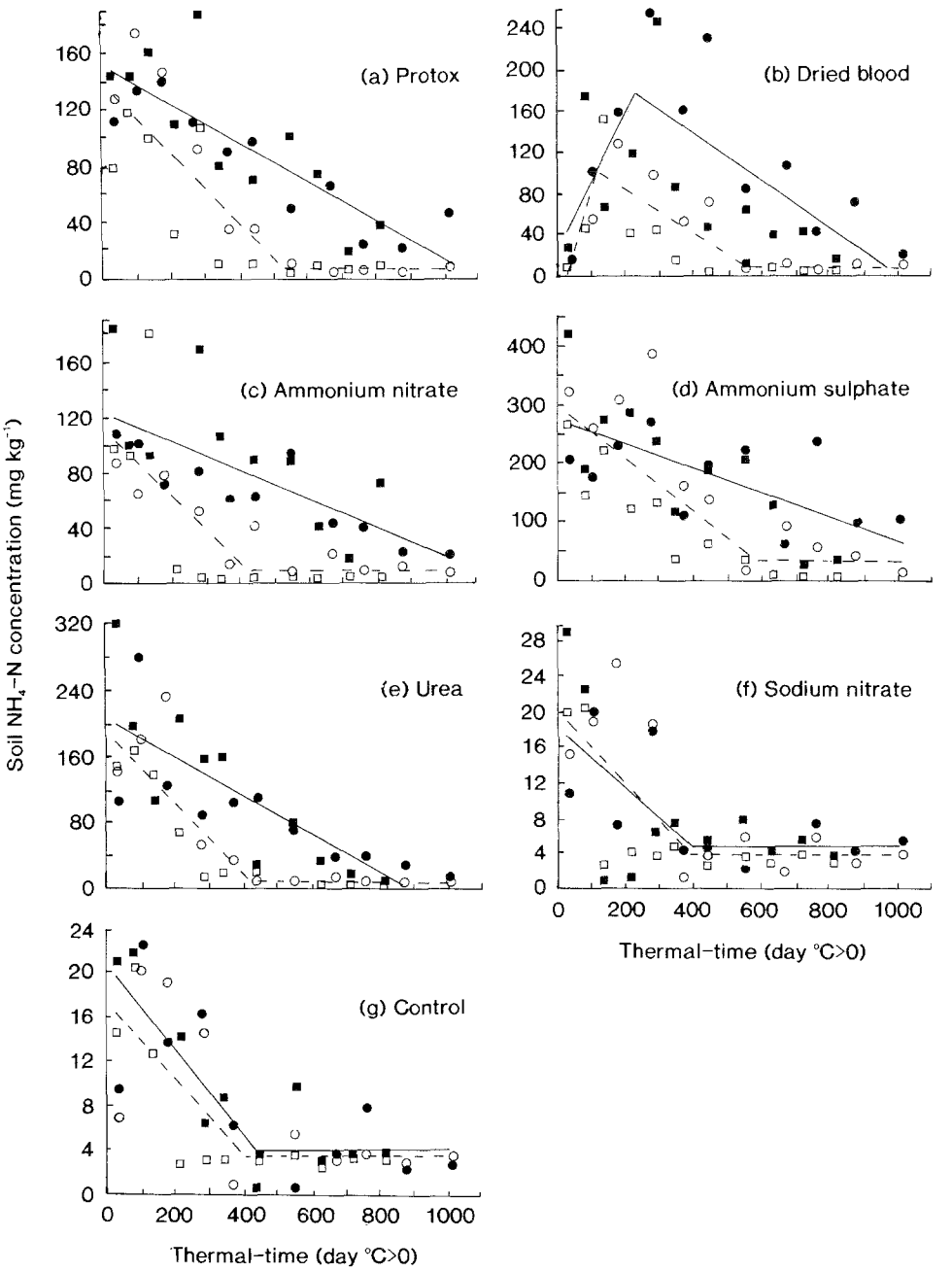

Fig. 1. Relationships between thermal-time (day°C > 0) and soil NH_4–N concentration (mg kg^{-1}) following application of various N fertilizers and nitrification inhibitor (□– – –□ without DCD, unprotected; ○– – –○ without DCD, protected; ■– – –■ with DCD, unprotected; ●– – –● with DCD, protected: symbols denote experimental values).

derived from mineralization of residual soil organic matter. Therefore, concentrations remained small as rates of mineralisation were low due to the recalcitrant nature of soil organic components.

The changing NO_3–N concentration in soil supplied with sodium nitrate is indicative of the dynamic nature of NO_3^- movement through the surface layers of soil by leaching following rainfall and irrigation, and capillary action resulting

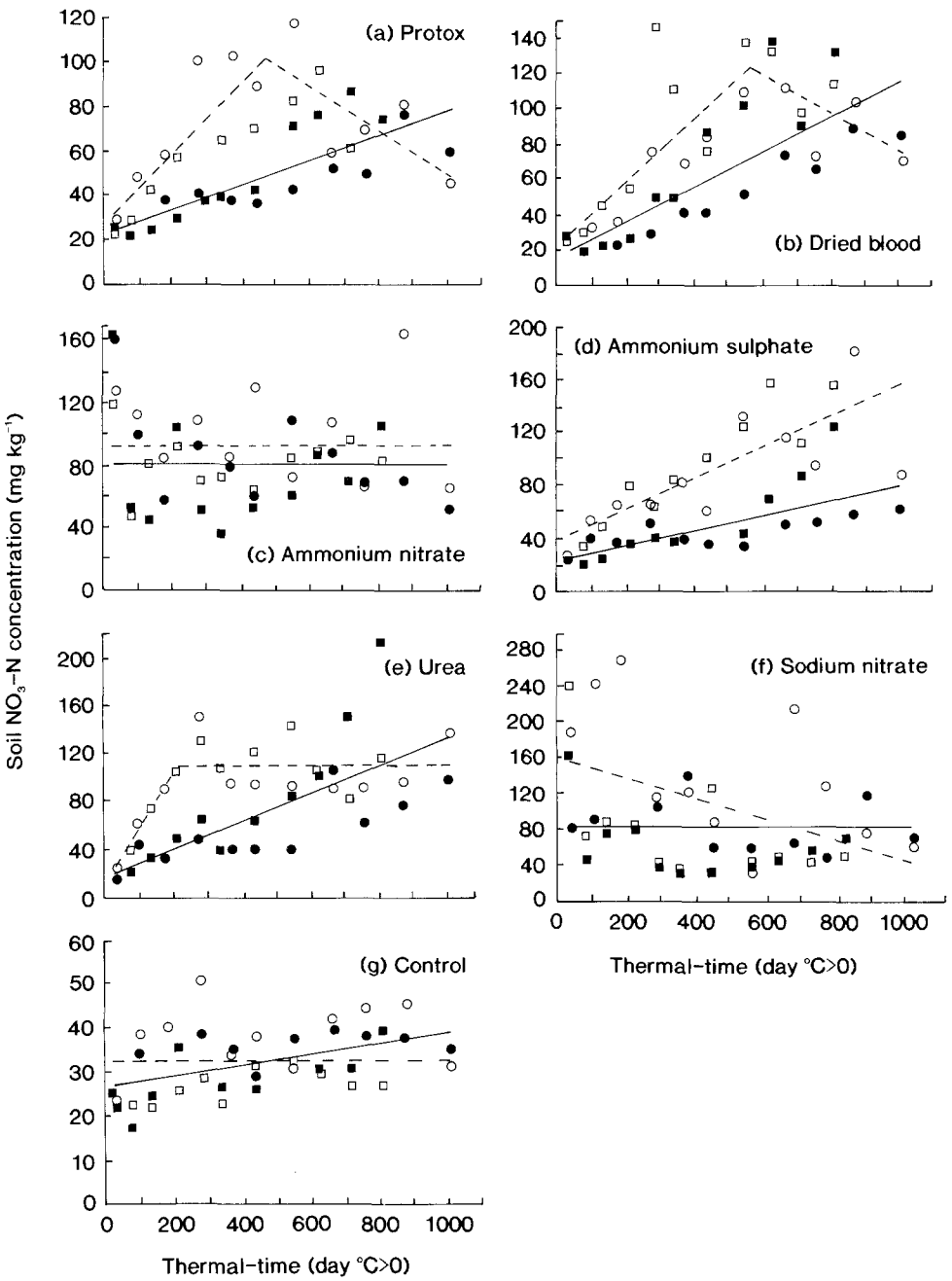

Fig. 2. Relationships between thermal-time (day°C > 0) and soil NO_3–N concentration (mg kg^{-1}) following application of various N fertilizers and nitrification inhibitor (□---□ without DCD, unprotected; ○---○ without DCD, protected; ■---■ with DCD, unprotected; ●---● with DCD, protected: symbols denote experimental values).

from moisture evaporation at the soil surface. However, it is unlikely that soil conditions encouraged substantial N losses by leaching or denitrification from the crop root zone, particularly at the protected site. Therefore, declining soil concentrations of NO_3–N towards the end of the experiment may be explained because available N was immobilised by the soil microbial population [21]. Other studies [20] have shown that as much as 60 kg N ha^{-1} can be lost from

soil within seven days, even when there is no waterlogging to encourage denitrification, or any evidence of leaching. Nitrate-N content of soil supplied with ammonium nitrate remained constant during the course of the experiment. This was probably because fertilizer NO_3^- removed from the soil was replaced by NO_3–N derived from nitrification of the NH_4^+ component of the material.

Crop N status

Summer cabbage is particularly effiicient at absorbing fertilizer N [9]. It follows, therefore, that total N content and N recovery of plants supplied with organic and inorganic N sources reflect total availability of fertilizer N over the crop duration. Nitrogen fertilizer application significantly increased the total N concentration in cabbage compared with the control, irrespective of the form supplied (Table 2). However, total N content of cabbage was generally smallest with organic N compared with mineral forms reflecting the lower availability of N from protein-based fertilizers. This was also supported by smaller recoveries of N by the crop from the organic sources although differences between the forms of N examined were not significant (Table 3). Unavailable fertilizer N is likely to increase the organic N status of soil following repeated applications, and be released to subsequent crops over a long period, thus potentially reducing N fertilizer requirements.

Total N content and N recovery by cabbage at the protected site were smaller compared with plants grown without protection. As leaching or denitrification were probably unimportant other mechanisms were responsible for the effects of environmental conditions on crop N status. Volatilization losses of NH_3 were probably also

Table 2. Total N concentration (% dry matter) in cabbage supplied with various N fertilizers and a nitrification inhibitor (DCD)

	Total N concentration (% dry matter)			
	Unprotected site			
N source	Without DCD	With DCD	Without DCD	With DCD
Protox	3.5	4.1	3.5	3.8
Dried blood	4.0	3.9	3.5	4.3
Ammonium nitrate	3.9	4.3	3.6	3.9
Ammonium sulphate	4.3	4.5	3.9	4.4
Urea	3.9	4.4	–	–
Sodium nitrate	4.1	4.3	3.7	3.8
Control	2.5	2.6	2.7	3.0
SED	0.25		0.45	

Table 3. Effect of N source and nitrification inhibitor (DCD) on the N recovery (%) by summer cabbage

	Nitrogen recovery (%)			
	Unprotected site		Protected site	
N source	Without DCD	With DCD	Without DCD	With DCD
Protox	27	31	20	21
Dried blood	34	27	23	26
Ammonium nitrate	36	39	26	23
Ammonium sulphate	37	38	26	33
Urea	32	43	–	–
Sodium nitrate	36	39	25	16
SED	ns[1]		ns	

[1]not significant

small as fertilizers were thoroughly incorporated into the soil which is known to limit these gaseous losses almost completely [8]. This is supported since total N content and N recovery of plants supplied with NH_4–N were similar to that with sodium nitrate. However, increased loss of gaseous N from plants grown in the warmer regime [28] and greater immobilization of fertilizer N could partly account for the lower recovery and N content in this condition compared with the cooler regime. A similar phenomenon was reported for lettuce grown under warm and cool glasshouse conditions [25]. In contrast, the total N content of cabbages grown without applied N was larger in the warmer condition. This may be explained because mineralization of residual soil organic matter can contribute more N to the plant when soil contains little mineral N [19] and since the rate of N release, and therefore N uptake, would be greater in the warmer condition.

Lairon *et al.* (1984) [15] showed that NO_3–N accumulation by lettuce supplied with organic N was smaller compared with mineral forms. Here, however, NO_3–N content of cabbage at the end of the experimental period was similar irrespective of the form of N supplied (Table 4). These contradictory results may be explained because Lairon *et al.* (1984) [15] grew lettuce in pots for a relatively short duration such that N was probably absorbed largely as NH_4^+. In this field study sufficient time was given for the mineralization and complete nitrification of the applied protein-based N such that the NO_3–N content of cabbage was similar to that with mineral forms. Large concentrations of unassimilated NO_3^- in harvested crops represents inefficient utilization of applied N, but can be avoided by correct N fertilizer application management.

Nitrification inhibition

Nitrification inhibitors may increase the efficiency of applied N fertilizer by maintaining N as NH_4^+ thus reducing the likelihood of NO_3–N loss through leaching and/or denitrification [22]. Here, DCD reduced the rate of nitrification and so increased the thermal-time required for NH_4–N depletion and NO_3–N accumulation in soil amended with NH_4–N forming materials (organic N, ammonium nitrate, ammonium sulphate and urea) compared with fertilizer alone (Fig. 1 and Fig. 2). However, the inhibition was not fully effective and, towards the end of the experiment, NO_3–N concentration in soil supplied with DCD approached that of soil without inhibitor. This was probably explained because rising soil temperatures increased the rate of degradation of the material which ultimately reduced its inhibitory activity [22]. Nevertheless, these results indicate that DCD slows the rate of NO_3–N accumulation in soil supplied with organic N sources and may effectively reduce potential NO_3^- leaching losses from these materials during crop establishment.

Dicyandiamide increased the total N content of cabbage (Table 2). Nitrification inhibitors generally raise plant N status by reducing NO_3–N leaching and denitrification losses [22]. However, little N was lost by these routes under

Table 4. Nitrate-N concentration (% dry matter) in summer cabbage supplied with various N fertilizers and a nitrification inhibitor (DCD)

	Nitrate concentration (%dry matter)			
	Unprotected site		Protected site	
N source	Without DCD	With DCD	Without DCD	With DCD
Protox	0.51	0.70	0.87	0.68
Dried blood	0.41	0.43	0.64	0.93
Ammonium nitrate	0.45	0.54	0.52	0.75
Ammonium sulphate	0.75	0.53	0.91	0.61
Urea	0.60	0.73	–	–
Sodium nitrate	0.60	0.84	0.90	1.07
Control	0.27	0.25	0.33	0.34
SED	0.10		0.25	

the experimental conditions reported here. Therefore, it is likely that differences in N absorption may be explained since DCD also acted as a slow-release N fertilizer [3] and contributed N to the plants which increased crop yield marginally (Table 1) but had no effect on N recovery (Table 3). Therefore, maintaining fertilizer N as NH_4^+ in soil may not necessarily increase the level of N recovery unless substantial quantities of NO_3–N are lost from the crop root zone by leaching or denitrification.

It has been suggested that nitrification inhibitors could be applied with NH_4–N fertilizers to reduce the NO_3–N content of vegetables [22]. However, since the inhibitory action of DCD declined over the duration of the crop, NO_3–N concentration in harvested plants was similar irrespective of the form of N supplied, and was larger than in controls (Table 4). Nitrate levels in harvested vegetables should be minimised by modifying fertilizer applications to suit crop requirements rather than through the use of nitrification inhibitors.

Fertilizer application and soil pH

Acidification of soil following application of inorganic NH_4–N has been frequently reported [1]. Here, for example, ammonium sulphate reduced the soil pH from an initial value of pH 7.0 to 5.2, 33 days after application to the soil. Organic N materials also reduced soil pH, but the acidifying effect was smaller than for ammonium sulphate, only decreasing to pH 6.5, which was comparable with that for urea and ammonium nitrate. Dicyandiamide reduced the acidifying effect of NH_4–N sources by decreasing the rate of nitrification. Therefore, the soil cation exchange system could more effectively buffer against the acidifying effect of the fertilizer materials under these conditions.

Soil microbial activity

Carbon dioxide concentration in soil air is a sensitive index of the activity of heterotrophic soil micro-organisms [14]. Dicyandiamide had no significant effect on CO_2 efflux and thus appeared to inhibit nitrification specifically without toxic action on other biological processes. Soil microbial activity increased with soil temperature and, generally with moisture content (Fig. 3). As in earlier studies [5, 14], temperature accounted for a larger proportion of the variation (up to 50% in some cases) in CO_2 concentration than moisture content. Kowalenko *et al.* (1978) [14] determined that CO_2 evolution from field soils was negatively correlated with water content at low soil temperatures. In contrast, Buyanovsky and Wagner (1985) [5] found increasing moisture content raised soil CO_2 concentration due to the combined effect of increased biological activity and reduced gas diffusion.

Increased CO_2 evolution from soils amended with organic N compared with the control is probably explained due to the enhanced activity of zymogenous micro-organisms [10] in response to greater substrate availability. In contrast to effects of organic N fertilizer on soil respiration, inorganic forms generally reduced microbial activity compared with the control, particularly at low soil moisture content. This deleterious response to mineral N application has been reported frequently and may occur due to reduced soil pH, direct inhibition by N compounds [13], reduced carbon availability through complex formation [11], and partial soil sterilization resulting from low osmotic potential [4]. In the work described here, there was no apparent effect of pH on soil respiration since microbial activity in soil supplied with, for example, sodium nitrate (which had little effect on pH) and ammonium sulphate (which significantly reduced pH) were similar. Increasing moisture content raised the level of microbial activity of soil amended with mineral N. Therefore, it seems likely that the inhibitory effect of mineral N was due to the reduced osmotic potential of the soil solution. No significant effect of soil moisture on microbial activity was detected with applied ammonium nitrate because the range of moisture contents experienced when CO_2 was measured did not include high moisture levels.

Mineral N replacement with activated sewage sludge derived protein

The productivity of modern intensive cropping systems mainly relies on large inputs of inorganic N fertilizers. However, increasing interest in

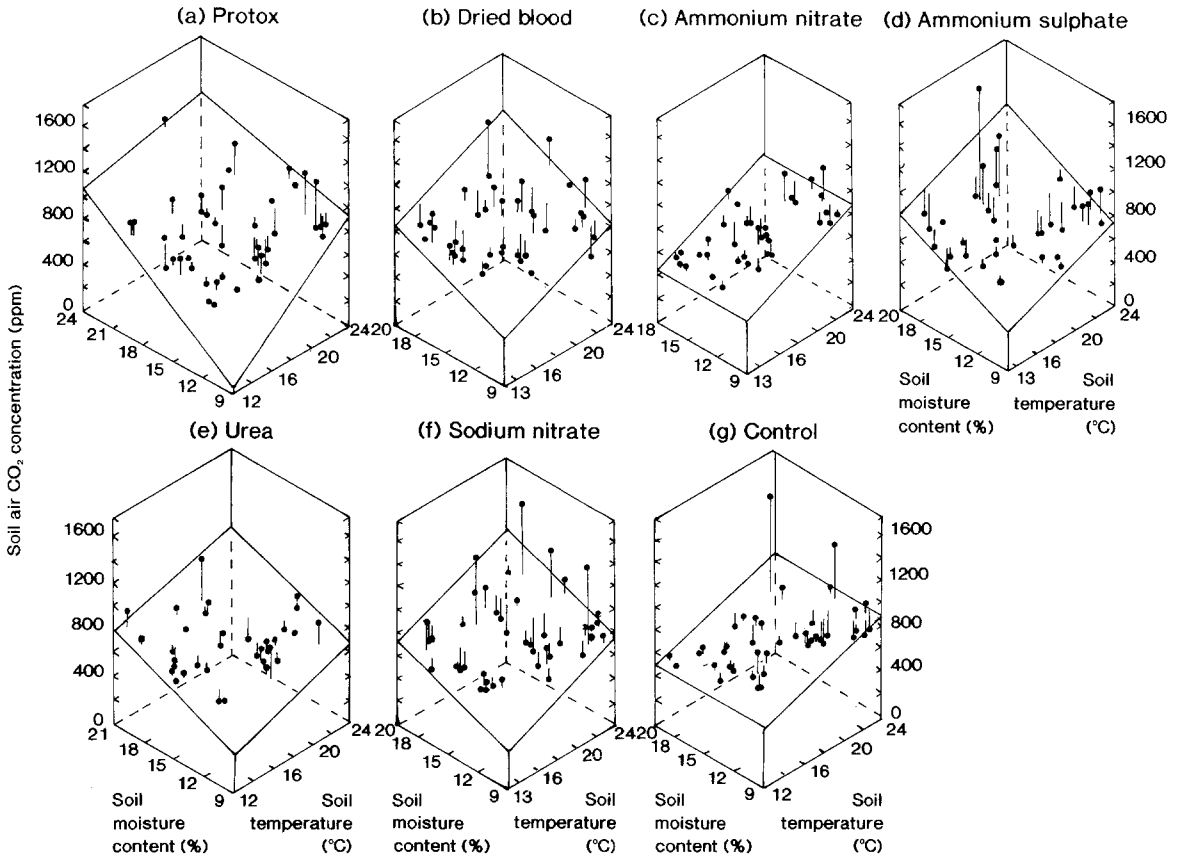

Fig. 3. Microbial activity in soil amended with various N fertilizers under contrasting soil temperature and moisture content conditions. Symbols denote experimental values.

novel sludge processing technology in the UK, and greater awareness of the environmental and economic benefits of recycling sludge products to agricultural land may potentially reduce the dependence on artificial nutrient sources. Activated sewage derived protein is a concentrated organic N material which gives consistent and reproducible crop yield responses and could therefore provide an effective alternative to conventional inorganic fertilizers for crop production.

Acknowledgements

The financial assistance of Oxfam is gratefully acknowledge. The authors wish to thank Miss T. Lee, Miss D. Livermore and Mr. G. Ruffles for their technical assistance.

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