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Arginine ammonification as a method to estimate soil microbial biomass and microbial community structure

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Abstract

Ammonium and nitrate production were measured at 2 h following addition of arginine solution (0.3 mg g^{-1} soil) to 13 unamended soils. The amounts of NH_4–N, NO_3–N and total inorganic N (i.e. NH_4–N + NO_3–N) produced were closely related to soil ATP content, biomass C measured by the fumigation–extraction method (FE) and CO_2 evolution measured by substrate-induced respiration (r = 0.83 to 0.91). The method is little, if at all, improved if both NH_4–N *and* NO_3–N are measured in the soil extracts rather than NH_4–N alone. Arginine mineralization appears to be a fast and rapid method for estimating soil microbial biomass. However, the method is invalid in acid soil and soils containing large amounts of readily decomposable substrates. Arginine mineralization was not selectively inhibited by cycloheximide and streptomycin so it is not possible to use this approach to estimate separately the fungal and bacterial biomass. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Arginine ammonification; Microbial biomass; Community structure

1. Introduction

Arginine is one of the 20 essential amino acids. It has three amino groups: $HOOCH_2CHCH_2CH_2$-$CH_2NHC(NH_2)_2$. Microorganisms catabolise arginine via one or more of four major pathways: (1) the arginine-urease or arginase-urea amidolyase pathway, (2) the arginine transmidinase pathway, (3) the arginine deiminase pathway, (4) the arginine decarboxylase pathway. Except in the arginine transmidinase pathway, ammonium is an end-product (Abdelal, 1979). The released ammonium can be quickly nitrified in soil (Mishra and Misra, 1991). Alef and Kleiner (1986) reported that more than 50 bacterial strains used arginine as a C and N source.

The arginine mineralized to ammonium during its decomposition can be readily extracted from soil and measured. Alef and Kleiner (1987a) suggested that, under specific conditions, the arginine ammonification rate was proportional to the amount of soil microbial biomass. Hund et al. (1988) attempted to use the arginine ammonification rate as an indicator of the effects of pollutants on microorganisms. Also, analogous to the substrate-induced respiration method (SIR) (Anderson and Domsch, 1978), arginine ammonification, coupled with selective inhibitors, might be useful in estimating the proportions of bacterial and fungal biomass in soil (Anderson and Domsch, 1973a,b,1975). The original method of Alef and Kleiner (1986) does not take account of NO_3–N production after arginine addition. If significant, this would underestimate and cause error in the estimation of total mineralization of arginine during the bioassay.

Our main aim was to attempt to improve the original arginine ammonification method of Alef and Kleiner (1987a). This was done by measuring the production of both NH_4–N and NO_3–N after arginine addition in a wide range of soils including acidic and

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Table 1
Soils

Soil No.	Location	Sample depth (cm)	Organic C (%)	Total N (%)	pH	Managements	Texture
1	Broadbalk nil plot 32	0–23	0.88	0.095	7.5	winter wheat	clay loam[f]
2	Broadbalk FYM plot 022	0–23	2.94	0.273	7.2	winter wheat	clay loam[f]
3	Broadbalk N_4PK plot 09	0–23	1.02	0.116	7.3	winter wheat	clay loam[f]
4	Broadbalk wilderness	0–10	4.42	0.401	6.7	forest	clay loam[f]
5	Highfield grassland	0–10	4.08	0.364	4.8	grass	clay loam[f]
6	Geescroft wilderness	0–10	6.65	0.389	3.2	forest	clay loam[f]
7	Geescroft fallow	0–23	2.17	0.183	4.9	fallow	clay loam[f]
8	Woburn long-term fallow	0–10	0.69	0.045	5.1	fallow	sandy loam[g]
9	Woburn ley-arable expt plot 32	0–10	1.21	0.120	6.4	grass ley[a]	sandy loam[g]
10	Woburn ley-arable expt plot 72	0–10	2.00	0.147	6.1	grass ley[b]	sandy loam[g]
11	Woburn ley-arable expt plot 75	0–10	1.75	0.144	6.2	clover/grass ley[c]	sandy loam[g]
12	Woburn ley-arable expt plot 67	0–10	1.14	0.109	6.0	winter bean[d]	sandy loam[g]
13	Woburn ley-arable expt plot 60	0–10	1.42	0.127	6.4	clover/grass ley[e]	sandy loam[g]

[f] Approximately 24% clay.
[g] Approximately 8% clay.
[a] From yr 1 of grass ley in a winter wheat; summer barley; 3 yr all grass ley rotation.
[b] From yr 8 of grass ley in a winter wheat; spring barley; 8 yr grass ley rotation.
[c] From yr 8 of clover/grass ley in a winter wheat; spring barley; 8 yr clover/grass ley rotation.
[d] From alternate winter wheat; spring barley; barley; barley; winter bean.
[e] From yr 2 of clover/grass ley in a winter wheat; spring barley; 3rd year clover/grass ley rotation.

substrate-amended ones, and correlating arginine mineralization with biomass C, soil ATP content and substrate-induced respiration. A further aim was to investigate the effects of selective inhibitors on arginine mineralization to see if this approach could (as with SIR) be used to assess microbial community structure.

2. Materials and methods

2.1. Soils

Thirteen soils were sampled from long-term field experiments at Rothamsted and Woburn, UK, using a 5 cm dia Dutch auger. Soils Nos. 1–3, 7 and 8 were from the 0–23 cm depth, the others were from the 0–10 cm depth. After sampling, the moist soils were sieved (2 mm). Plant residues and fauna (e.g. earthworms) were removed by hand. The moist soils were adjusted to 40% of full water-holding capacity (WHC) and then incubated at 25°C for 7 d in a container with water and soda-lime. The conditioned (preincubated) soils were stored in a cold room (5°C) prior to biomass measurements.

A small portion of the soils was air-dried and then ground (< 160 μm) for soil chemical analysis. Soil organic C was determined by the dichromate digestion method (Kalembasa and Jenkinson, 1973). Soil total N was measured by automated thermal combustion (Europa Scientific Co.). Soil pH was measured with a pH meter using a 1:1 ratio of moist soil to water. Details of the soil characteristics are given in Table 1.

2.2. Ryegrass amendment

Portions of moist soil Nos. 3, 5, 6, 8–10 (1 kg) were amended with finely-ground ryegrass (< 1 mm) which contained 39.8% C and 2.5% N (2% w/w). A small volume of distilled water was added with a syringe to adjust the water contents of the soils to 50% WHC. The amended soil samples were then incubated in glass vials at 25°C for 10 d in a container which also contained water and soda-lime.

2.3. Fumigation and incubation

Portions of the same soils were fumigated with alcohol-free $CHCl_3$ at 25°C for 24 h, and then incubated at 25°C for 10 d as described above following removal of $CHCl_3$.

2.4. Microbial biomass measurements

Biomass C was measured by the fumigation–extraction method (FE) (Vance et al., 1987b; Wu et al., 1990) using moist soils containing 25 g oven-dry (o.d.) soil. Soil ATP content was determined by the method of Jenkinson and Oades (1979) as modified by Tate and Jenkinson (1982). All measurements were done in triplicate.

2.5. Substrate-induced respiration (SIR)

The SIR rate was determined by a modified West and Sparling (1986) method (Lin and Brookes, 1996). Briefly, three portions of moist soil were weighed into

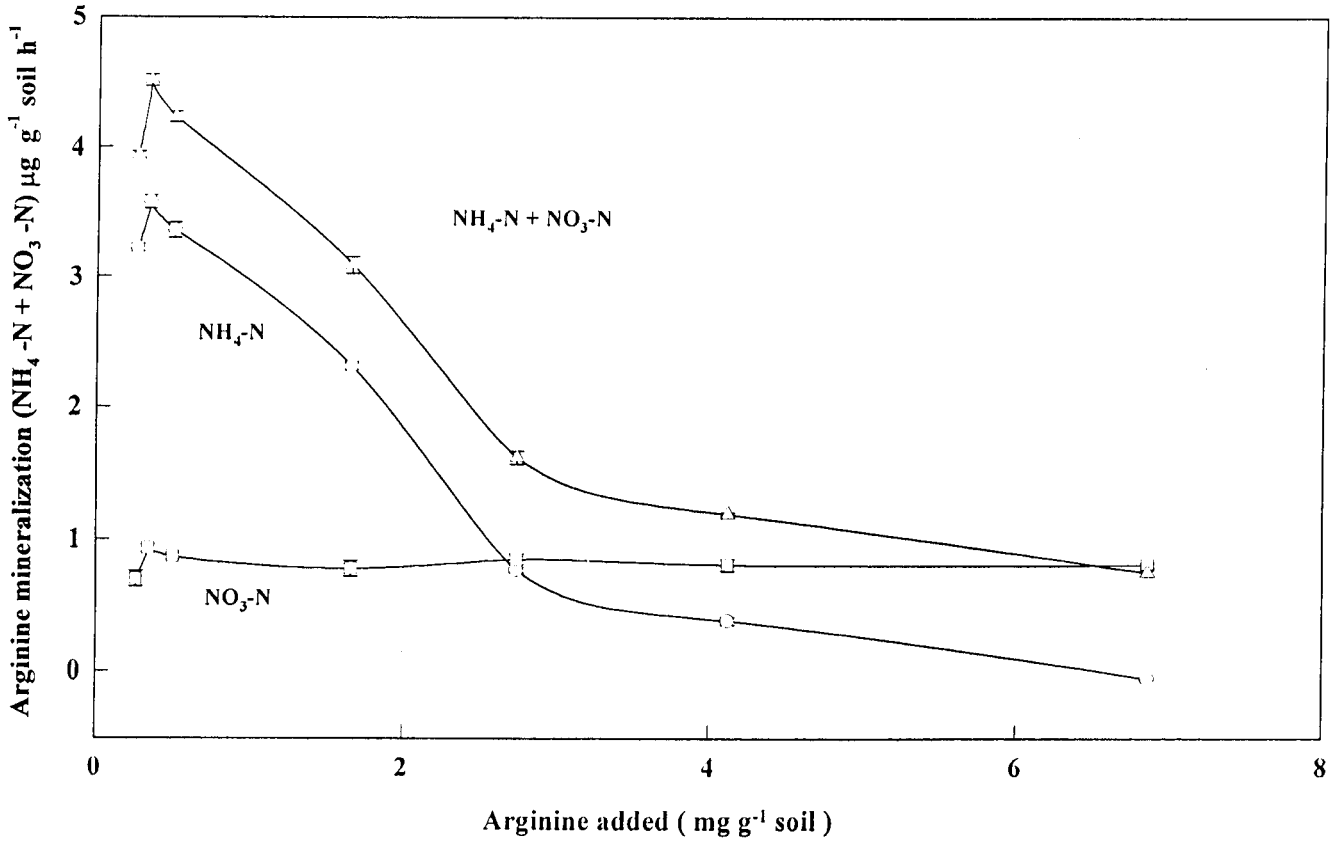

Fig. 1. Mineralization of arginine added at different rates and incubated for 1 h at 25°C (soil No. 9). Bar is standard deviation.

Quickfit flasks and then mixed thoroughly with glucose solution (6 mg glucose g^{-1} soil). The glucose solution increased soil moisture to 1.2-fold WHC (1.0 = 100%). After 30 min the soil samples were kept at 25°C for 2 h (with shaking at 200 rpm). Carbon dioxide in the headspace of the flask was sampled with a syringe and measured by gas chromatography. A calibration to account for CO_2 dissolved in the soil solution was done for all soils apart from the acid soil No. 6.

2.6. Basic procedures for measuring arginine mineralization

The approach was basically that of Alef and Kleiner (1987a), except that soil weights etc were increased as were all other reagents proportionately. Briefly, for each determination, three portions of moist soil (40% WHC), each containing the equivalent of 10 g o.d. soil, were weighed into 125 ml plastic bottles, amended with arginine solution of different concentrations (0.26, 0.34, 0.50, 1.66, 2.74, 4.12 and 6.86 mg g^{-1} soil) and then adjusted to 2.0-fold WHC. They were then incubated at 25°C for up to 6 h with or without shaking at 150 rpm. The bottles were then frozen at −15°C for at least 4 h. The soil samples were then thawed in hot water and immediately extracted with 2 M KCl (1:4 soil to solution ratio) for 15 min by shaking at 200 rpm. After filtration (Whatman No. 42) the soil extracts were stored at −15°C prior to analysis. The initial soil NH_4–N and NO_3–N concentrations were also determined at the time the measurement of arginine mineralization began. The amounts of NH_4–N and NO_3–N in the soil extracts were measured separately by an automatic NH_4^+ and NO_3^- analyzer (Alpken RFA/2) and arginine ammonification and total arginine mineralization rates (μg g^{-1} soil h^{-1}) determined.

2.7. Measurement of selective inhibition on arginine mineralization

Three portions of moist Highfield soil, each containing 10 g o.d. soil, were amended with (a) arginine solution (0.3 mg g^{-1} soil, 1:2 soil-to-water ratio), (b) arginine + streptomycin (0.5–6 mg g^{-1} soil) solution, (c) arginine + cycloheximide (2–16 mg g^{-1} soil) solution and (d) arginine + streptomycin + cycloheximide. The amended soil samples were incubated and then extracted with 2 M KCl. Ammonium and nitrate in the soil extract were determined as described above.

Table 2
The interference of arginine with the measurement of NH_4–N and NO_3–N in KCl solution

Added arginine conc.	Measured	
	NH_4–N (mg l^{-1})	NO_3–N (mg l^{-1})
0	4.3	4.1
50	4.0	4.1
100	2.9	4.1
200	2.1	4.1
500	0.5	4.1
2000	0.0	4.1
LSD (p = 0.05)	2.91	0.12

3. Results and discussion

3.1. Response of microorganisms to arginine addition

Moist soil No. 9 was amended with a series of seven concentrations of arginine solution and incubated at 25°C without shaking. The production of NH_4–N and NO_3–N was determined at 1 h after arginine addition (Fig. 1).

The concentration of added arginine greatly affected the rates of both NH_4–N and NO_3–N production. The highest mineralization rates, 3.6, 0.9 and 4.5 μg g^{-1} soil h^{-1} for NH_4–N, NO_3–N and total inorganic N (NH_4–N + NO_3–N), respectively, were obtained at the low addition rate of 0.34 mg arginine g^{-1} soil. It apparently decreased rapidly at higher rates of arginine addition. Total inorganic N mineralized from arginine decreased from 4.5 to 0.8 μg (NH_4–N + NO_3–N) g^{-1} soil h^{-1} with the arginine addition increasing from 0.34 to 6.86 mg g^{-1} soil. No NH_4–N production could be determined at the addition rate of 6.86 mg arginine g^{-1} soil. However, NO_3–N production did not significantly change with increasing arginine addition.

Ammonium was determined by an automated indophenol procedure, which is based on the Berthelot colour reaction (Searcy et al., 1965). In this method, ammonium reacts with salicyclate and hypochlorite in a buffered alkaline solution in the presence of sodium nitroferricyanide (pH 12.8–13) to form the salicylate acid analog of indophenol blue. The blue–green colour production is measured at 660 nm. White and Gosz (1981), Burton et al. (1989) and Searle (1990) found that amino acids present in KCl solution interfered with the measurement of NH_4–N by the automated indophenol procedure. We added a series of concentrations of arginine to 2 M KCl solution which contained NH_4^+ and NO_3^-. The measured concentration of ammonium decreased apparently linearly with increasing arginine concentration (Table 2). No NH_4–N could be detected at 2000 mg arginine l^{-1} in KCl sol-

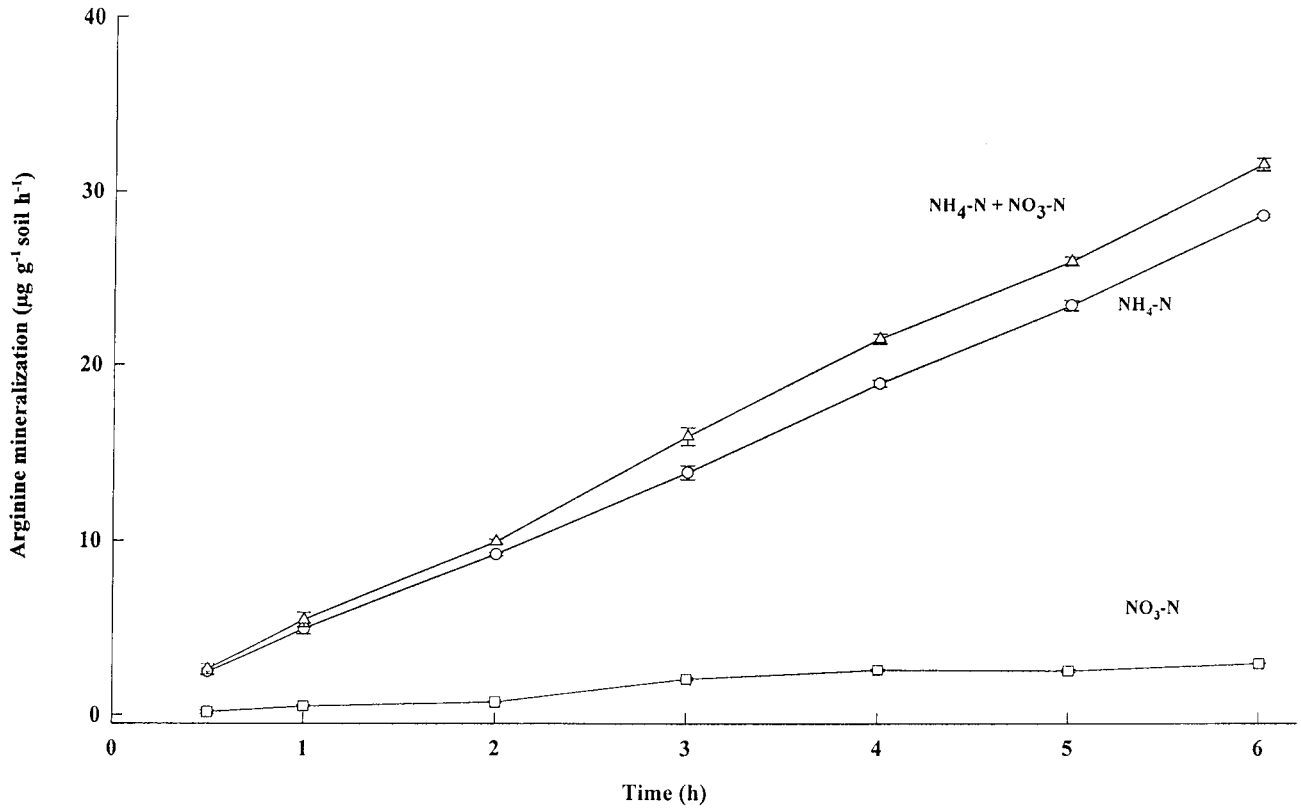

Fig. 2. Arginine mineralization during incubation (soil No. 9). Bar is standard deviation.

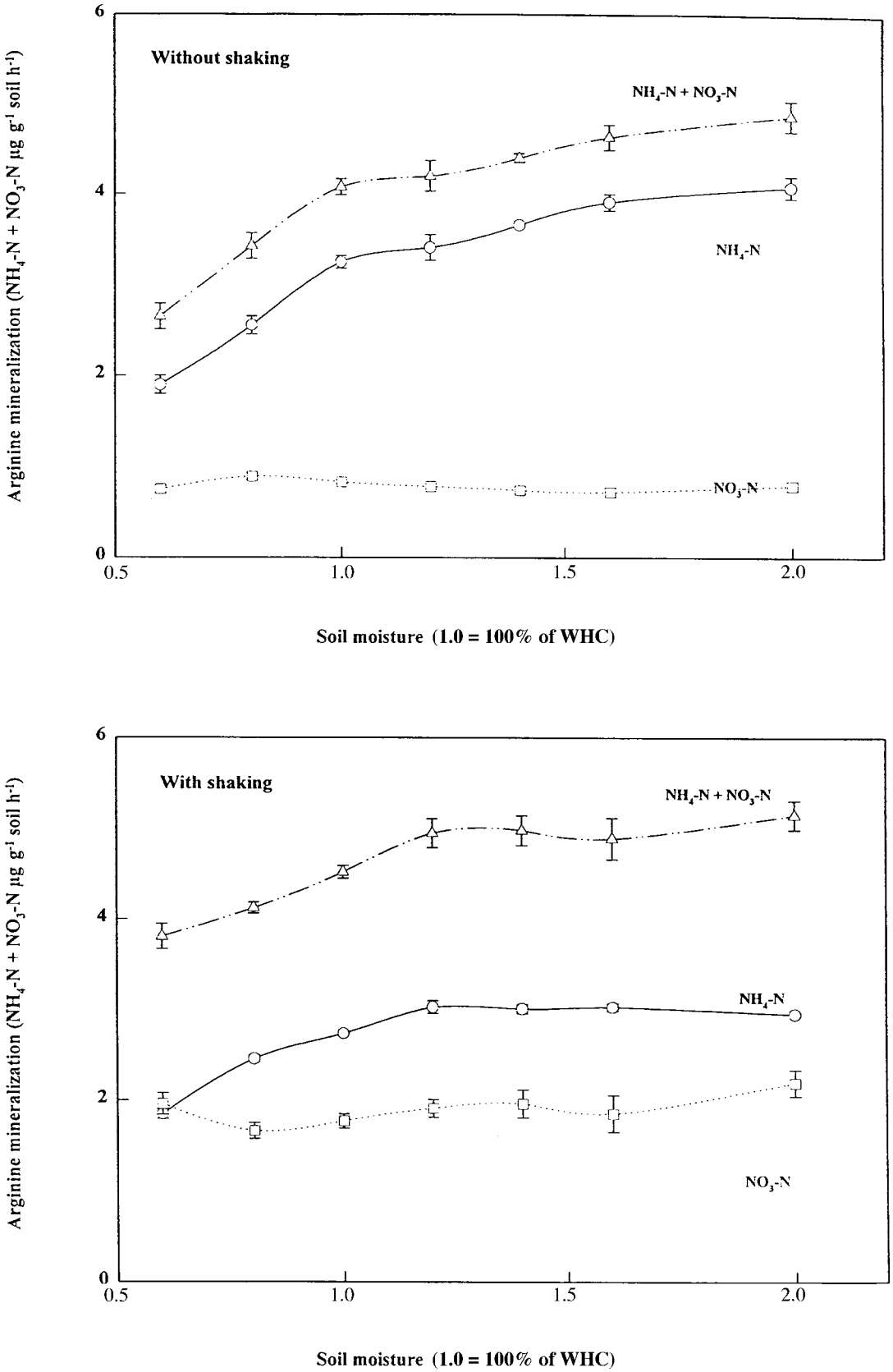

Fig. 3. The effects of soil moisture on arginine mineralization (soil No. 9). Bar is standard deviation.

Table 3
Initial mineral N, K_2SO_4-extractable C and arginine mineralization in unamended soils

Soil No.	Initial mineral N			K_2SO_4-extractable carbon ($\mu g\ g^{-1}$ soil)	Arginine mineralization		
	NH_4–N ($\mu g\ g^{-1}$ soil)	NO_3–N ($\mu g\ g^{-1}$ soil)	(NH_4–N + NO_3–N) ($\mu g\ g^{-1}$ soil)		NH_4–N ($\mu g\ g^{-1}$ soil h^{-1})	NO_3–N ($\mu g\ g^{-1}$ soil h^{-1})	(NH_4–N + NO_3–N) ($\mu g\ g^{-1}$ soil h^{-1})
1	0.2 ± 0.01[a]	2.5 ± 0.02	2.7 ± 0.03	36 ± 2.3	1.5 ± 0.01	1.5 ± 0.01	3.0 ± 0.02
2	0.9 ± 0.01	20.6 ± 0.65	21.5 ± 0.66	200 ± 2.7	6.5 ± 0.10	3.4 ± 0.02	9.9 ± 0.12
3	0.2 ± 0.01	10.7 ± 0.35	10.7 ± 0.36	48 ± 0.7	3.6 ± 0.03	1.8 ± 0.01	5.3 ± 0.04
4	0.6 ± 0.01	73.2 ± 3.25	73.7 ± 3.26	237 ± 3.0	17.1 ± 2.65	6.7 ± 1.00	23.7 ± 3.65
5	1.6 ± 0.02	42.1 ± 2.61	43.7 ± 2.63	137 ± 3.2	10.0 ± 0.35	5.2 ± 0.05	15.2 ± 0.40
6	2.4 ± 0.05	43.1 ± 3.65	45.5 ± 3.70	219 ± 2.8	1.2 ± 0.01	1.4 ± 0.01	2.5 ± 0.02
7	0.6 ± 0.03	26.3 ± 3.20	26.9 ± 3.23	71 ± 2.1	2.2 ± 0.10	1.2 ± 0.10	3.3 ± 0.20
8	0.1 ± 0.00	1.8 ± 0.01	1.9 ± 0.01	6 ± 0.1	0.1 ± 0.00	0.0 ± 0.01	0.1 ± 0.00
9	0.5 ± 0.01	8.9 ± 0.32	9.4 ± 0.33	43 ± 2.3	3.8 ± 0.25	1.1 ± 0.06	4.9 ± 0.31
10	1.6 ± 0.02	8.5 ± 0.95	10.1 ± 1.04	48 ± 1.4	7.1 ± 0.34	2.0 ± 0.12	9.1 ± 0.46
11	1.7 ± 0.01	11.9 ± 0.65	13.6 ± 0.66	49 ± 1.4	5.4 ± 0.35	0.7 ± 0.01	6.1 ± 0.36
12	1.5 ± 0.01	9.6 ± 0.39	11.1 ± 0.40	34 ± 1.3	0.3 ± 0.01	1.0 ± 0.01	1.3 ± 0.02
13	1.9 ± 0.02	3.9 ± 0.12	5.7 ± 0.14	41 ± 2.2	2.1 ± 0.12	1.2 ± 0.06	3.3 ± 0.18

[a] Standard deviation.

ution. However, the measurement of NO_3–N was not influenced at any rate of arginine addition.

In the original method, ammonium was measured by Fawcett and Scott's (1960) procedure. A large amount of arginine would remain in the extracts of soils of low biomass content and interfere with the measurement of NH_4–N as shown above. Thus, the best results were obtained by using a small amount of arginine (0.30 mg g^{-1} soil) and a longer incubation, at least 2 h. Our results therefore generally confirm the original technique of Alef and Kleiner (1986,1987a).

3.2. The time-course of NH_4–N and NO_3–N production after arginine addition

Moist soil No. 9 was amended with 0.3 mg arginine g^{-1} soil and adjusted to 2.0-fold WHC. The amended soil was incubated at 25°C for 6 h and the production of NH_4–N and NO_3–N determined at 1 h intervals after arginine addition (Fig. 2).

Alef and Kleiner (1986) reported that the rate of arginine ammonification remained linear for at least 1 h. Our results concurred; rates of NH_4–N and NO_3–N production following arginine addition did not change over 6 h at 25°C incubation. This suggests that the microorganisms mineralized arginine without changing their activity during this short incubation period. This constant rate also occurs with the measurement of substrate-induced respiration which implies that the same is true.

3.3. The effects of water content on NH_4–N and NO_3–N production following arginine mineralization

Moist soil No. 9 was amended with different volumes of arginine solution to provide from 0.6 to 2.0-fold WHC and a constant arginine addition rate of 0.30 mg arginine g^{-1} soil. The amended soil was incubated at 25°C without or with shaking at 150 rpm, and the production of NH_4–N and NO_3–N was measured at 1 h after arginine addition (Fig. 3).

Ammonium-N production, measured without shaking during incubation, increased rapidly from 1.9 to 3.3 μg NH_4–N h^{-1} g^{-1} soil from 0.6 to 1.00-fold WHC, then increased slowly up to a maximum of 4.4 μg NH_4–N h^{-1} g^{-1} soil at 2.0-fold WHC. Nitrate production was not significantly affected by water content. With shaking during incubation, NH_4–N production increased from 1.9 to 3.0 μg NH_4–N h^{-1} g^{-1} soil from 0.6 to 1.2-fold WHC, but not as rapidly as without shaking, and then remained constant. Nitrate-N production was not influenced by water content either. However, more than twice as much NO_3–N was found with shaking than without, whilst only 60% of the NH_4–N produced without shaking was measured when the soil was shaken during incubation. This was due to nitrification of NH_4^+ to NO_3^- during incubation with shaking, presumably due to higher O_2 availability. Total inorganic N production (NH_4–N + NO_3–N) was quite similar without or with shaking (Fig. 3).

3.4. Relationships between arginine mineralization and soil properties

Ammonium-N mineralized from arginine ranged from 0.1 to 17.1 μg NH_4–N g^{-1} soil h^{-1}; NO_3–N produced from nitrification of NH_4^+ ranged from 0.0 to 6.7 μg NO_3–N g^{-1} soil h^{-1}; the total inorganic N (NH_4–N + NO_3–N) thus ranged from 0.1 to 23.7 μg g^{-1} soil h^{-1}. An arable soil from the Broadbalk

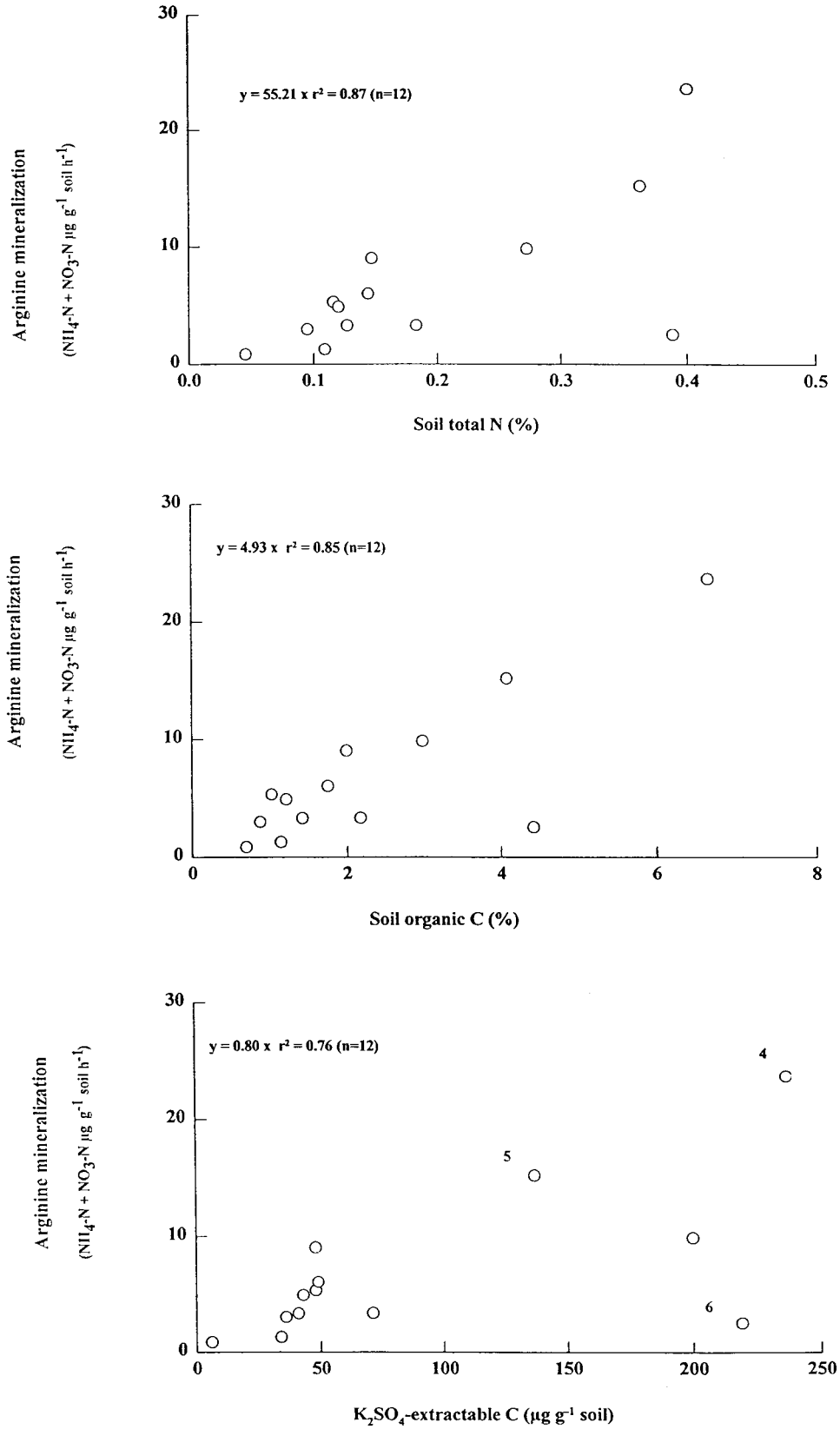

Fig. 4. Relationship between arginine mineralization and soil organic C, K_2SO_4-extractable C and total N (excluding soil No. 6).

Table 4
The correlations between arginine mineralization and biomass C (Bc), ATP and substrate induced respiration (SIR) in thirteen soils

		Coefficient (r)
Biomass C	Bc (μg g^{-1} soil) = 63.3 (±9.47) μg g^{-1} soil (NH_4–N)	0.89
	Bc (μg g^{-1} soil) = 166.5 (±21.29) μg g^{-1} soil (NO_3–N)	0.91
ATP	ATP (nmol g^{-1} soil) = 0.5 (±0.07) μg g^{-1} soil (NH_4–N)	0.89
	ATP (nmol g^{-1} soil) = 1.1 (±0.21) μg g^{-1} soil (NO_3–N)	0.83
SIR	SIR (μl CO_2 evolved g^{-1} soil h^{-1}) = 3.9 (±0.55) μg g^{-1} soil (NH_4–N)	0.90
	SIR (μl CO_2 evolved g^{-1} soil h^{-1}) = 9.6 (±1.61) μg g^{-1} soil (NO_3–N)	0.86

Continuous Wheat Experiment which had received inorganic fertilizer (soil No. 3) had a lower arginine mineralization rate than another soil from the same experiment which received farmyard manure (soil No. 2), presumably a reflection of the smaller biomass in the former case, 234 and 423 μg biomass C g^{-1} soil, respectively (Table 3).

Alef and Kleiner (1987a) reported that arginine ammonification rates ranged from 0.51 to 13 μg NH_4–N h^{-1} g^{-1} soil in their 34 soils, comparable to our results. However, Kaiser et al. (1992) reported much lower rates. In their 27 soils, NH_4–N production following arginine addition ranged from 0.03 to 2.71 μg g^{-1} soil h^{-1}. However, in neither case did they take account of the NO_3–N produced.

We found no relationship between arginine mineralization rate and either soil pH or clay content. Alef and Kleiner (1986,1987a), Alef et al. (1988) and Kaiser et al. (1992) also found similar results. However, both reported high correlations between arginine ammonification rate and soil organic C and total N content. In our work, 13 soils covering a wide range of pH (3.2 to 7.5) (Wu, 1990), managements and organic C content (Table 1) were used. No significant correlations were found between the arginine mineralization rate and either soil total organic C content, K_2SO_4-extractable C or soil total N content when all of the soils were used in the statistical analysis. However, the correlations were highly significant when an acidic soil (soil No. 6) was excluded from the statistical analysis (Fig. 4). The correlation coefficients (r) were then 0.92, 0.87 and 0.93 for organic C, K_2SO_4-extractable C and total N respectively.

Thirty-four soils were used in Alef and Kleiner's (1987a)study. One of them contained 8% organic C, and its inclusion greatly decreased the significance of the correlations. Kaiser et al. (1992) used 27 soils. Two of them contained more than 16% organic C. They did not report the influence of these two soils on the correlations. In our work, soils Nos. 4 and 5 contained more than 4.0% organic C. They did not apparently affect the correlations. Soil No. 6 contained not only 6.65% organic C, but also had a very low pH (3.2). The low pH is the most likely reason for the aberrant behaviour of this soil. This is discussed below.

3.5. Correlations between arginine mineralization and biomass carbon, soil ATP content and substrate-induced respiration

Alef and Kleiner (1987a) found a significant correlation between the NH_4–N mineralized from arginine and O_2 uptake after glucose addition ($O_2 = 3.22$ NH_4–N). Alef and Kleiner (1987b) reported that the arginine ammonification rate was closely correlated with the amount of soil microbial biomass. Alef et al. (1988) also reported high correlations between NH_4–N production following arginine addition and soil ATP content, heat output and biomass C measured by SIR. However, Kaiser et al. (1992) found that the relationship was relatively poor between the quantity of NH_4–N due to arginine addition and the extra C extracted following fumigation with chloroform. Wilke (1989) also reported that arginine ammonification was not a good indicator of the effects of inorganic pollutants on soil N transformation. In our work, highly significant correlations existed between the (NH_4–N) and (NH_4–N plus NO_3–N) produced following addition of arginine and the amounts of biomass carbon, soil ATP content and CO_2 evolution due to glucose addition (SIR) (Table 4 and Fig. 5). However, while we expected that use of (NH_4–N + NO_3–N) rather than (NH_4–N) alone, in providing an index of *total* arginine mineralized, would improve the method, in practice it did not. Firstly, there was no better correlation with (NH_4–N + NO_3–N) than (NH_4–N) alone with other indicators of microbial activity. Secondly, the loss in precision caused by the usually high background NO_3–N makes it difficult to measure the comparatively small amount of NO_3–N mineralized from arginine. Thirdly, there is considerably more analytical work.

The regression equations between arginine mineralization following arginine addition and SIR rates: SIR = 3.9 (NH_4–N) (r = 0.90, Table 4), were comparable to that between O_2 uptake following glucose addition and NH_4–N production due to arginine addition (Alef and Kleiner, 1987a). There is obviously a relationship between O_2 consumed and CO_2 evolved during respiration by aerobic organisms. By analogy, it would seem logical to assume that similar populations of microorganisms mineralized arginine and

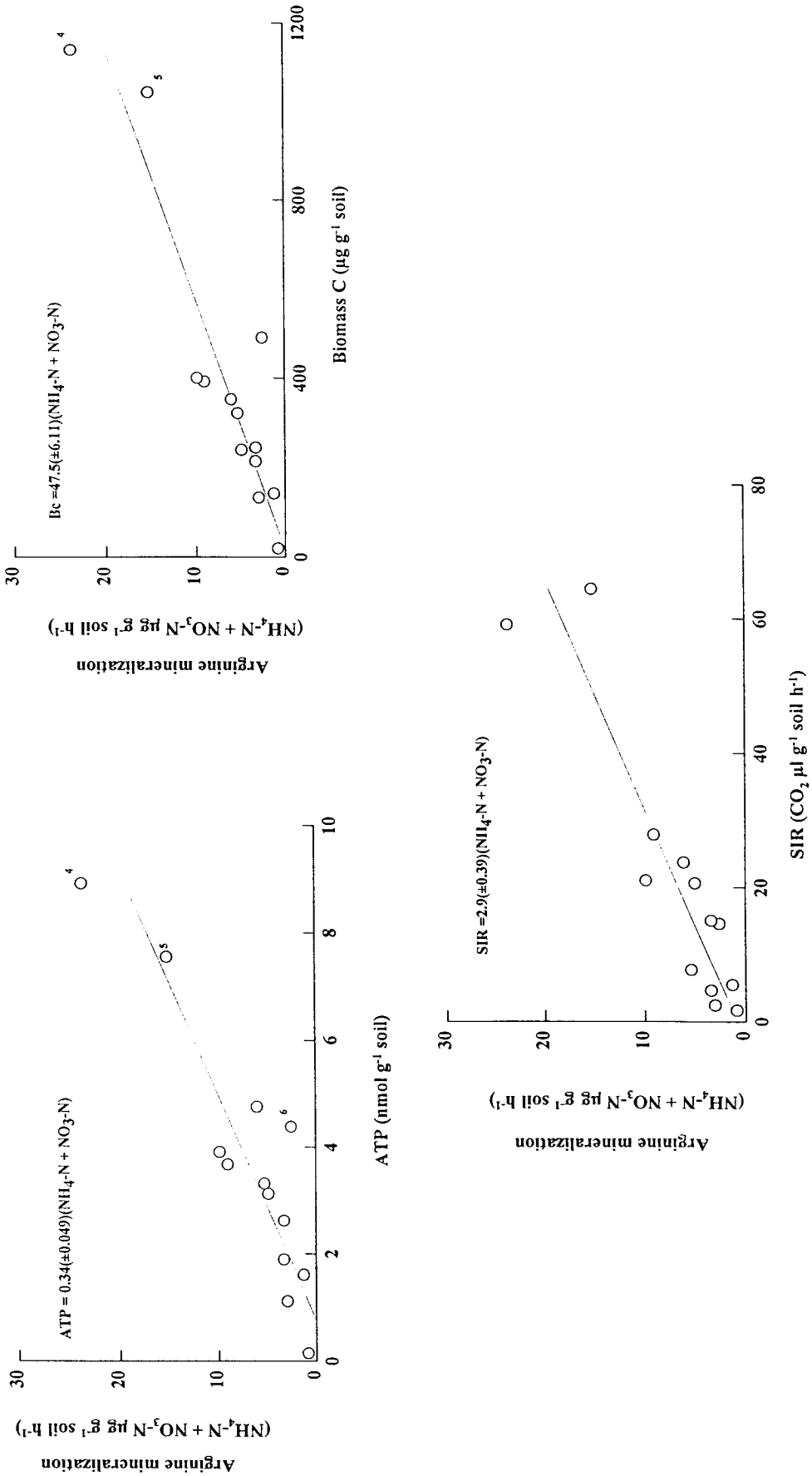

Fig. 5. Relationships between arginine mineralization and biomass C, soil ATP content and CO_2 evolution following addition of aqueous glucose.

Table 5
The correlation coefficients between arginine mineralization (NH_4–N + NO_3–N) and biomass C, ATP and SIR

	Coefficient (r)[a]	Coefficient (r)[b]	Coefficient (r)[c]
Bc	0.91	0.96	0.92
ATP	0.89	0.96	0.82
SIR	0.90	0.91	0.78

[a] Soil No. 6 was excluded from the calculation (n = 12).
[b] Soil Nos. 4–6 were excluded from the calculation (n = 10).
[c] Soil Nos. 4 and 5 were excluded from the calculation (n = 11).

glucose. Furthermore, this is consistent with the hypothesis that all of these methods measure a similar population within the entire microbial biomass.

Alef and Kleiner (1987a) suggested that arginine ammonification was inhibited in soils which contained much readily available substrate and those with a high ammonium content. No inhibition of arginine mineralization was apparent when the initial NH_4–N content was less than 3 μg NH_4–N g^{-1} soil (Alef and Kleiner, 1987a). In our soils, initial native NH_4–N ranged from 0.1 to 2.4 μg NH_4–N g^{-1} soil, soil NO_3–N from 1.8 to 73.2 μg g^{-1} soil and organic C from 0.69 to 6.65% (Tables 1 and 3). Nitrate concentration did not affect arginine mineralization. One of Alef and Kleiner's (1987a) soils contained 8% organic C. Its inclusion decreased the correlation between arginine ammonification rate and O_2 uptake following glucose addition. In our work, soils Nos. 4 to 6 contained 4 to 6.65% organic C. The correlations between arginine mineralization rate and biomass C, soil ATP content and SIR were not significantly influenced by the omission of soils Nos. 4 and 5. However, the correlations were considerably improved when soil No. 6 was excluded from the statistical analysis (Table 5). It was very interesting that the correlation between arginine mineralization rate and CO_2 evolution following addition of aqueous glucose was not significantly influenced by this soil. This is consistent with the above hypothesis that soil of low pH has a different relationship between arginine mineralization rate and total organic C, K_2SO_4-extractable C and total N. This implies that relationships between biomass and its activity are relatively unaffected by decreasing soil pH. In contrast, the biomass concentration is decreased as pH falls. However, this needs confirmation with more soils before it is proven.

In both the substrate-amended and fumigated soils, arginine mineralization was very low (Table 6). The main reason was presumably because of microbial immobilization of NH_4–N during the decomposition of organic C from ryegrass or the fumigant-killed biomass. This suggests that arginine mineralization, unlike SIR, cannot be used as an estimate of microbial biomass in soils which are immobilizing inorganic N during the early phase of substrate decomposition.

3.6. Inhibition of arginine mineralization by antibiotics

Ammonium and nitrate production decreased rapidly when aqueous solutions giving equivalent to 0.5 to 2 mg streptomyin g^{-1} soil was added. Both then decreased slowly to zero at 6 mg streptomycin g^{-1} soil (Fig. 6). A similar pattern was also found with cycloheximide addition. Arginine mineralization decreased rapidly when aqueous solutions given 2 to 8 mg cycloheximide g^{-1} soil was added to moist soil. It then decreased slowly to zero at 16 mg cycloheximide g^{-1} soil. Thus the addition rates of 3 mg streptomycin and 10 mg cycloheximide g^{-1} soil were used in an attempt to measure the contributions of bacterial and fungal biomass to total arginine mineralization. Thus we attempted to extend Anderson and Domsch's (1978) selective inhibition method for quantifying the fungal and bacterial components of the microbial biomass.

Table 6
Arginine mineralization in the ryegrass-amended soils and fumigated-incubated soils

Soil No.	Arginine mineralization					
	Ryegrass-amended[a]			Fumigated-incubated[b]		
	NH_4–N (μg g^{-1} soil h^{-1})	NO_3–N (μg g^{-1} soil h^{-1})	NH_4–N + NO_3–N (μg g^{-1} soil h^{-1})	NH_4–N (μg g^{-1} soil h^{-1})	NO_3 (μg g^{-1} soil h^{-1})	NH_4–N + NO_3–N (μg g^{-1} soil h^{-1})
3	7.3 ± 0.56	−8.0 ± 0.84	−0.7 ± 1.40	−2.2 ± 0.12	0.1 ± 0.01	−2.3 ± 0.13
5	24.6 ± 2.95	0.2 ± 0.01	2.8 ± 2.96	−2.5 ± 0.25	−0.4 ± 0.06	−2.9 ± 0.31
6	−20.3 ± 3.65	−6.9 ± 1.32	−27.3 ± 4.97	−11.5 ± 3.65	−2.0 ± 0.28	−13.5 ± 3.93
8	−1.1 ± 0.05	−1.9 ± 0.10	−3.1 ± 0.15	−0.8 ± 0.01	−0.0 ± 0.00	−0.8 ± 0.01
9	7.3 ± 0.68	3.5 ± 0.74	10.7 ± 1.42	−2.6 ± 0.12	−0.1 ± 0.01	−2.7 ± 0.13
10	10.0 ± 2.13	−5.1 ± 0.32	4.9 ± 2.35	−16.5 ± 4.31	0.1 ± 0.01	−16.3 ± 4.32

[a] Measurement was made 10 d after ryegrass amendment.
[b] Measurement was made at 10 d incubation following removal of $CHCl_3$.

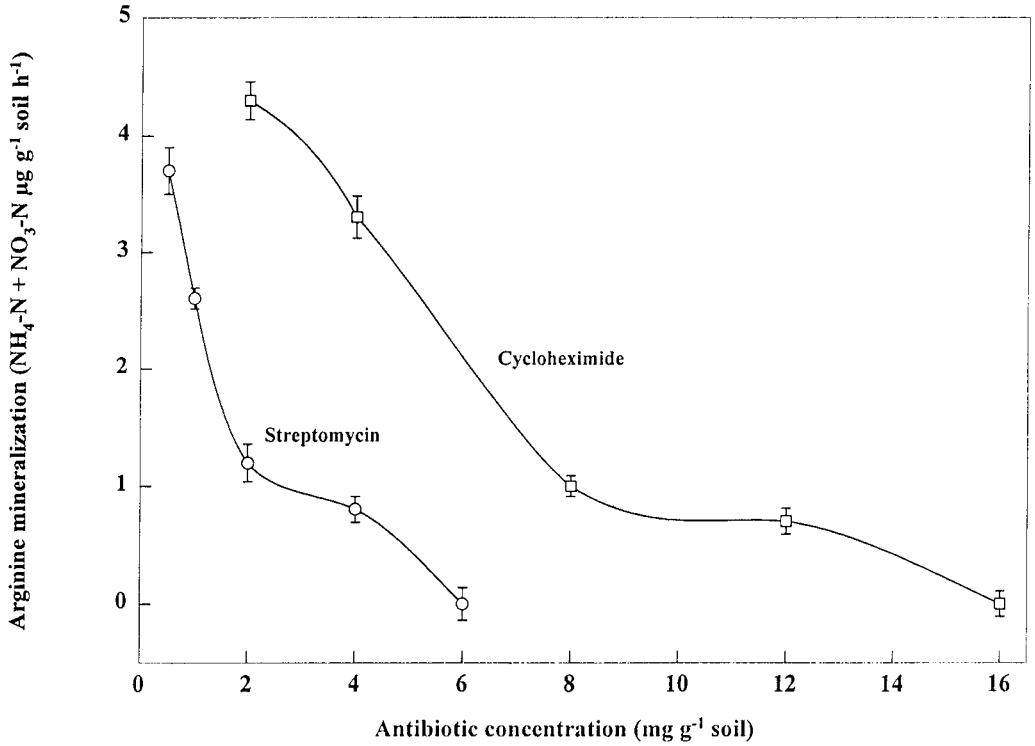

Fig. 6. Relationship between inhibition of arginine mineralization and concentrations of antibiotics added to Highfield soil. (Bar is standard deviation).

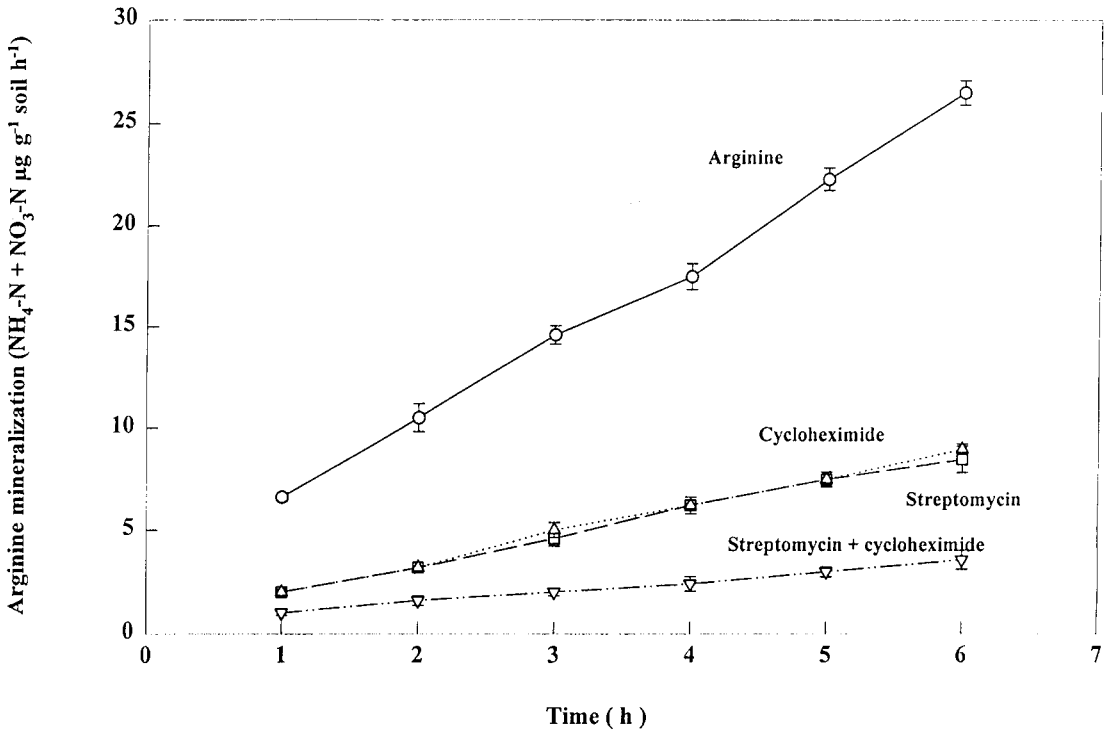

Fig. 7. Inhibition of arginine mineralization by antibiotics (Highfield grassland soil). (Bar is standard deviation).

Arginine mineralization was inhibited to a similar extent (about 70%) during the assay time of 6 h by both cyclohexamide (10 mg g^{-1} soil) and streptomycin (3 mg g^{-1} soil) added separately (Fig. 7). However, the total combined inhibition was only 80% when both inhibitors were added in combination to the soil. The synergistic effect $[(a-b)+(a-c)]/(a-d))$ was very large, more than 1.5. (Where a is the NH_4–N and NO_3–N production following addition of arginine only; b is that following addition of arginine + streptomycin; c is that following addition of arginine + cycloheximide and d is that following addition of arginine + streptomycin + cycloheximide). It was clear that both inhibitors did not selectively inhibit arginine mineralization. This is contrary to the hypothesis that both arginine mineralization and the SIR method measure the same fraction of soil microbial biomass. There are two possible explanations. The first could be that the antibiotics depress arginine mineralization through a different mechanism to that involved in glucose mineralization. Secondly and most likely, the reason could be that arginine mineralization and the SIR method each measure a slightly different fraction of the soil microbial biomass. This difference may not be very important relative to the generally large biomass content in most of the soils used. The relationship between those methods for estimating total microbial biomass is thus not significantly influenced. More work is required to understand the behaviour of the antibiotics on arginine mineralization. However, it does not seem likely that selective inhibition of arginine mineralization could provide a valid measure of the proportions of fungi and bacteria in the soil microbial biomass.

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