



# Failure to simulate C and N mineralization in soil using biomass C-to-N ratios as measured by the fumigation extraction method?

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## Abstract

A C and N mineralization model (DETRAN) was used to simulate C and N dynamics in an unfertilized soil (NIL plot) and a soil annually fertilized with organic (FYM plot) or inorganic fertilizer (NPK plot). The soils amended with or without rye, i.e. 500 mg C and 30 mg N kg<sup>-1</sup> dry soil (D.S.), were incubated for 180 d at 25°C and microbial biomass C and N, CO<sub>2</sub> production and inorganic N (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>) were monitored. The production of CO<sub>2</sub> was greater in the NIL plot than in the NPK plot but three times lower than in the FYM plot. The soil microbial biomass C and N decreased in the FYM soil but not in the NIL and NPK plots. The N mineralization was 7.5 times greater in the FYM plot than in the NPK and NIL plots but the application of rye had no significant effect on it. In the unamended soil, an efficiency for C of 40%, i.e. the amount of C incorporated into the microbial biomass while the rest or 60% evolved as CO<sub>2</sub>, had to be used to link the C and N dynamics in the NPK and NIL plots but the efficiency was only 26% in the FYM plot. The efficiency for C added with the rye was 45% in the NIL plot and 60% in the NPK plot but it was difficult to link C and N mineralization in the FYM plot.

We conclude that the dynamics of C and N as governed by the microbial biomass C and N were different for the FYM plot compared with the NPK and NIL plots. The difference, a result of long-term organic fertiliser application, could be due to a difference in efficiency for C, a difference in the C-to-N ratio of the microbial biomass; a difference not reflected in the measured values, or/and a difference in the dynamics of N. © 2000 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

Models can be developed to simulate, predict or test concepts of processes. We developed the DETRAN model to test concepts of C and N dynamics, e.g. C and N mineralization (Dendooven, unpublished Ph.D. thesis, K.U. Leuven, 1990) and the denitrification process (e.g. Dendooven et al., 1994). The part of the DETRAN model simulating C and N mineralization is based on models developed by Paul (1984) and van Veen et al. (1985) and considers three main pools: applied organic material, soil organic matter and mi-

crobial biomass. Each of these pools is further divided into fractions; three for the added organic material and soil organic matter and two for soil microbial biomass.

We report here on the simulation of C and N mineralization in soil (NIL plot) fertilized annually with or without inorganic fertilizer (NPK plot) or farmyard manure (FYM plot) and amended with or without rye, i.e. 500 mg C and 30 mg N kg<sup>-1</sup> dry soil (D.S.). The soils were incubated for 180 d at 25°C and microbial biomass C and N, CO<sub>2</sub> production and inorganic N (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>) were monitored. The model was used to investigate (i) possible effects of annual application of organic or inorganic fertilizer on C and N mineralization and microbial biomass C and N dynamics and (ii) possible effects of application of rye.

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## 2. Materials and methods

### 2.1. Experimental site

The study was carried out with soil from section 1 of the Broadbalk Continuous Wheat experiment at Rothamsted in Southwest England (for details see Johnston and Garner, 1969). The soil is classified as a Stagnogleyic paleo-argillic brown earth belonging to the Batcombe soil series, with a loamy surface layer overlying Clay-with-flints (Avery and Catt, 1995). The soil is classified as an Aquic paleudalf under the USDA system for soil taxonomy and the particle size distribution is 284, 246, 268, 137 and 64 g kg<sup>-1</sup> for the particles <2, 2–20, 20–50, 50–200, 200–2000 µm, respectively (Avery and Bullock, 1968).

### 2.2. Soil sampling

Soil was sampled within the plough layer (0–23 cm) on 3 November 1994 from plot 031 that has received no fertilizer since 1843 (referred to as the NIL plot), plot 081 amended with 144 kg N ha<sup>-1</sup> yr<sup>-1</sup> (as 'Nitram' which contains 38% N as NH<sub>4</sub>NO<sub>3</sub>), 35 kg P ha<sup>-1</sup> yr<sup>-1</sup> and 90 kg K ha<sup>-1</sup> yr<sup>-1</sup> (referred to as the NPK plot) and from plot 221 amended with 35 t farm-yard manure ha<sup>-1</sup> yr<sup>-1</sup> (referred to as the FYM plot): this supplied on average 248 kg N, 43 kg P and 325 kg K (for more details see Dyke et al., 1983). The NIL plot had an organic C and N content of 8.7 g kg<sup>-1</sup> dry soil (D.S.) and 1.01 g kg<sup>-1</sup> D.S. while it was 10.4 g kg<sup>-1</sup> D.S. and 1.22 g kg<sup>-1</sup> D.S. in the NPK and 30.2 g kg<sup>-1</sup> D.S. and 2.96 g kg<sup>-1</sup> D.S. in the FYM plot. The pH was 8.1, 7.2 and 7.7 in the NIL, NPK and FYM plot, respectively.

### 2.3. Rye

Rye (growth stage 30 or ear at 1 cm approximately (Zadok et al., 1974)) including the crown, the main seminal and some fine roots were sampled on 16 March 1994, dried at 50°C for 5 d and hammer milled. The root-to-shoot ratio was 0.49 and the particle size distribution of the ground rye was 320, 350, 320 and 10 mg g<sup>-1</sup> on a dry matter basis (D.M.) for the particles <150, 150–250, 250–500 and 500 µm, respectively. The organic C and N content was 354.6 and 23.9 g kg<sup>-1</sup> D.M. The rye contained 144 g hemicellulose kg<sup>-1</sup> D.M., 98 g cellulose kg<sup>-1</sup> D.M., 64 g soluble carbohydrate kg<sup>-1</sup> D.M., 31 g lignin kg<sup>-1</sup> D.M. and 108 g kg<sup>-1</sup> total ash. The cellulose, hemicellulose and lignin content was determined using an ANKOM fibre analyser (New York). Total ash content was determined by loss on ignition while water-soluble carbohydrates in water extracts of herbage, as described by

Thomas (1976), in which neocuprin and copper sulphate were used instead of anthrone.

### 2.4. Aerobic incubation

The three soil samples were passed through a 6.5 mm sieve, adjusted to 40% of water holding capacity (WHC) and conditioned for 7 d in drums that contained one vessel with distilled water to keep the soil moist and another with 1 M NaOH to trap CO<sub>2</sub> evolved.

Thirty three sub-samples of 90 g of soil from each plot were amended with or without 92 mg of rye and placed in 160 ml glass containers. This procedure resulted in concentrations of approximately 500 mg C kg<sup>-1</sup> and 30 mg N kg<sup>-1</sup> being added to the soil. At the onset of the experiment, three glass containers were selected at random from each treatment and used for measurements of water content, water holding capacity, microbial biomass and inorganic N content (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>). A sub-sample of 30 g of soil was extracted for inorganic N with 120 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub> solution; shaken for 30 min and filtered through Whatman No 42 paper. NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were measured in the extracts of the soil samples by an automated procedure according to the method of Crooke and Simpson (1971) and Hendriksen and Selmer-Olsen (1970), respectively. Additionally, three sub-samples of 30 g of soil were fumigated with ethanol-free chloroform in the dark for 24 h (Müller et al., 1992), extracted with 120 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub> solution; shaken for 30 min and filtered through Whatman No 42 paper. Organic C in the unfumigated and fumigated soil extracts was measured using a Dohrman DC80 carbon analyzer (Wu et al., 1990) and the organic N by potassium persulphate oxidation (Cabrera and Beare, 1993). Microbial biomass C and N was calculated as the amount of C or N released through fumigation, i.e. the difference between the organic C or N in the fumigated and non-fumigated samples, multiplied by 2.22 (Wu et al., 1990; Jenkinson, 1988, respectively). These provided zero time results.

The containers were placed in 1 l jars containing a vial with 20 ml of 1 M NaOH solution to trap CO<sub>2</sub> evolved and a vial with 10 ml distilled H<sub>2</sub>O to avoid desiccation during incubation. The jars were sealed airtight and incubated aerobically at 25°C for 180 d. After 1, 3, 7, 14, 28, 56, 84, 112, 140 and 180 d, three jars were selected at random, opened and the vessel containing 20 ml of 1 M NaOH solution was removed for analysis of CO<sub>2</sub>. The glass container was removed from the jar and the water content, microbial biomass C and N and the inorganic N concentrations were measured in the soil as mentioned earlier. The CO<sub>2</sub> trapped in 1 M NaOH solution was measured by auto-titration (Jenkinson and Powlson, 1976). Every 2

weeks, all remaining jars were opened, aired for 5 min, resealed and further incubated at 25°C.

### 2.5. Statistical analysis

CO<sub>2</sub> production was regressed on elapsed time using a linear regression model which was forced to pass through the origin but allowed different slopes (production rates) for each site (SAS Institute, 1989). This approach was based upon the idea that no CO<sub>2</sub> is produced at time zero. Production of NO<sub>3</sub><sup>-</sup> was analyzed in the same way, but the regression lines were not forced to pass through the origin and were allowed different intercepts as concentrations were not zero at time zero.

NO<sub>3</sub><sup>-</sup> and CO<sub>2</sub> production rates were subjected to a one way analysis of variance to test for significant differences between the plots and treatments. All analyses were performed using SAS statistical analysis (SAS Institute, 1989).

### 3. Model structure

DETRAN is a numerical model with a time step of 1 h written in FORTRAN77. It contains two main parts: one simulating the C and N mineralization and the other the reduction of N oxides. The part simulating the denitrification process is discussed in detail in Dendooven et al. (1994). The part of the DETRAN model simulating the C and N mineralization is based on models developed by Paul (1984) and van Veen et al. (1985) and considers three main pools: applied organic material, soil organic matter and microbial biomass.

Applied organic material was considered to contain three fractions: active, resistant and stable. The active fraction contains the soluble part of the cell and consists mainly of non-polymeric carbohydrates and proteins and is assumed to be readily available for microbial biomass (van Veen and Paul, 1981). The resistant fraction is assumed to be less decomposable and contains cellulose and hemicellulose or 24% of the added rye. If we assume that 45% of this fraction was C and given that the C content of the rye was 35.46% then this fraction contained 154 of the 500 mg C added. The stable or the most resistant fraction contained lignin or 3% of the added rye and assuming that 71% of this fraction was C then it contained 31 of the 500 mg C added. The ash of the applied organic material was assumed to be inaccessible for decay. The active fraction was assumed to be the total organic C minus the hemicellulose+cellulose fraction and the lignin fraction or 315 of the 500 mg C added and contained also all N added. Each of these fractions has a decay rate as derived from literature but adjusted

through a curve-fitting procedure (Dendooven and Anderson, 1995): the decay for the active fraction being the mean of decay-rates for glucose and a wide range of amino-acids. It was assumed that a proportion of decomposed organic C i.e. 60% (Payne, 1970) was incorporated into the microbial biomass while the rest evolved as CO<sub>2</sub>.

Soil organic material was also considered to contain three fractions: active, resistant and stable. The characterization of each of these fractions is far less well defined than for plant material. The active fraction includes crop and root residues and soil microbial metabolites or extra-cellular material. The resistant fraction consists mainly of humic material and is sometimes defined as the non-hydrolyzable (6 N HCl) component of soil organic matter (Martel and Paul, 1974). The stable fraction consists of two sorts of organic material one chemically stabilized (Jenkinson and Rayner, 1977) or the humus fraction (McGill et al., 1981) and one physically stabilized between clay layers. The size and turnover rates of the soil organic matter for the unamended soil were derived from literature (van Veen et al., 1985) and optimized through a curve-fitting of the data obtained in the aerobic incubation experiment (Dendooven and Anderson, 1995). The size and turnover rates that gave the best fit for the unamended soil were used to simulate the dynamics of soil organic matter when rye was added.

The microbial biomass contained two distinct fractions: an active and a stable one. The active fraction of the soil microbial biomass consists of those micro-organisms that respond immediately to the application of active and resistant organic material, decompose part of dead microbial biomass tissues and have a high death rate (van Veen et al., 1985). The stable fraction is the microbial biomass fraction that decomposes the lignin part of the added organic material and resistant and stable soil organic matter and has a lower death rate (van Veen et al., 1985). The size and turnover rates of the microbial biomass for the unamended soil were derived from literature (van Veen et al., 1985) and optimized through a curve-fitting of the data obtained in the aerobic incubation experiment (Dendooven and Anderson, 1995). The size and turnover rates that gave the best fit for the unamended soil were used to simulate the dynamics of microbial biomass when rye was added.

The amount of ammonified N was the result of decomposed N (determined by the organic material C-to-N ratio) minus assimilated N (determined by the C-to-N ratio of the microbial biomass).

The dynamics of C were simulated first and the values that resulted in the lowest residual sum of squares were used to simulate the N dynamics. It has to be stressed that the values for the different parameters obtained through this iterative curve-fitting

procedure were not unique, especially considering the large amount of parameters used in the model and the lack of possibility to link fitted with measurable values, e.g. the sizes of the two microbial pools and their turnover rates. They only resulted in 'a' best fit and small differences in the residual sum of squares as a measure of the differences between the measured and fitted values can be the result of a substantial difference in the value of the parameters used. Dendooven and Anderson (1995) discussed that in more detail when simulating the denitrification process.

## 4. Results and discussion

### 4.1. Production of $\text{CO}_2$ in the unamended soil

The  $\text{CO}_2$  production was significantly smaller in the NIL plot than the FYM plot but significantly greater than in the NPK plot ( $P < 0.05$ ) (Fig. 1a). The organic C content ( $10.4 \text{ g C kg}^{-1}$ ) and the soil microbial biomass C ( $183 \text{ mg C kg}^{-1}$ ) in the NPK plot were, however, greater than in the NIL plot,  $8.7 \text{ g C kg}^{-1}$  and  $147 \text{ mg C kg}^{-1}$ , respectively. One would thus expect a greater  $\text{CO}_2$  production in the NPK plot compared with the NIL plot. A possible explanation for this phenomenon was that there was less ground cover in the NIL plot than in the NPK plot so a larger popu-

lation of weeds grew in this plot (but not in the NPK) and their roots presumably provided an input of decomposable organic C substrate.

The  $\text{CO}_2$  was simulated satisfactorily and the model gave a near perfect fit (Fig. 1a). Characteristics of the different fractions used in the model are given in Table 1. van Veen et al. (1985) found similar characteristics for the stable soil organic matter fraction, i.e. 90% of total soil organic C with a decomposition rate of  $8 \times 10^{-7}$ . The decay rates for the active and resistant fractions they reported,  $0.8 \text{ d}^{-1}$  and  $0.3 \text{ d}^{-1}$ , however, were greater than values we found, e.g. in the NIL plot they were  $0.02 \text{ d}^{-1}$  and  $0.0019 \text{ d}^{-1}$ , respectively (Table 1).

### 4.2. Biomass C in the unamended soil

The microbial biomass C was greatest in the FYM plot and smallest in the NIL plot (Fig. 2a). The decrease in soil microbial biomass during the 180 d incubation was substantial in the FYM plot. It decreased with  $0.213\% \text{ d}^{-1}$  comparable with a value of  $0.228\% \text{ d}^{-1}$  reported by Joergensen et al. (1990) for soil from a permanent grass plot on Highfield, Rothamsted. There was no significant change in biomass C in the other plots.

The simulated biomass C values were characterized by a small increase followed by a small decrease: the magnitude of change being greater in the FYM plot. That increase was presumably caused by handling of the soil.

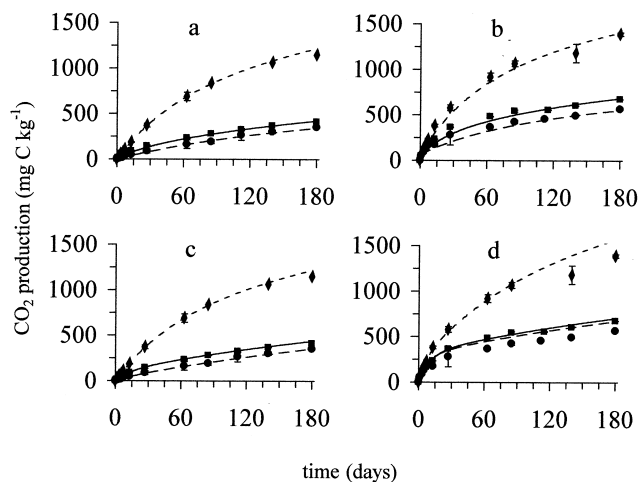


Fig. 1. Measured  $\text{CO}_2$  production ( $\text{mg C kg}^{-1} \text{ D.S.}$ ) from the unfertilized NIL plot (■), the NPK plot annually fertilized with  $144 \text{ kg N ha}^{-1}$  (●) and the FYM plot annually amended with  $35 \text{ t farmyard manure ha}^{-1}$  (◆) and aerobically incubated for 180 d at  $25^\circ\text{C}$ . (a) Simulated (lines) and measured (symbols) values for the NIL (—), NPK plot (---) and FYM plot (---) not amended with rye and assuming an efficiency of 60 and an adjusted microbial biomass C-to-N ratio for the unamended soil and (b) as (a) but for soil amended with  $1022 \text{ mg rye kg}^{-1} \text{ D.S.}$ , (c) as (a) but assuming an adjusted efficiency and using the measured microbial biomass C-to-N ratio for the unamended soil (d) as (c) but for soil amended with  $1022 \text{ mg rye kg}^{-1} \text{ D.S.}$  Bars indicate  $\pm 1$  standard deviation.

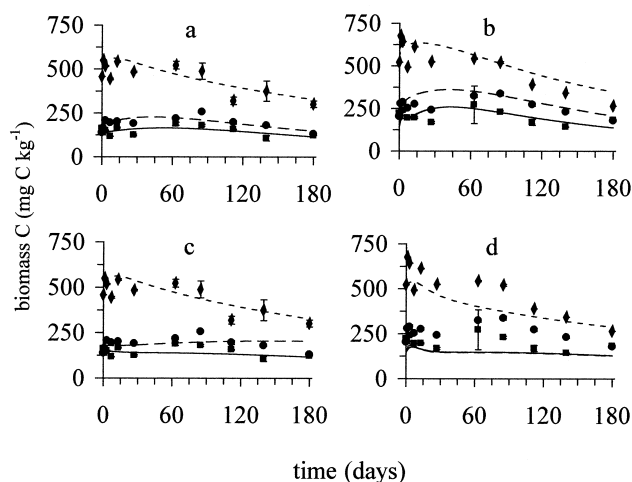


Fig. 2. Microbial biomass C ( $\text{mg C kg}^{-1} \text{ D.S.}$ ) from the unfertilized NIL plot (■), the NPK plot annually fertilized with  $144 \text{ kg N ha}^{-1}$  (●) and the FYM plot annually amended with  $35 \text{ t farmyard manure ha}^{-1}$  (◆) and aerobically incubated for 180 d at  $25^\circ\text{C}$ . Legends to the simulated values can be found in Fig. 1. Bars indicate  $\pm 1$  standard deviation.

Table 1  
Parameters obtained from an iterative curve fitting procedure of the unamended soil from section 1 of the Broadbalk Continuous Wheat experiment at Rothamsted resulting in the lowest residual sum of squares<sup>a</sup>

			Efficiency of 60 and adjusted microbial biomass C-to-N ratio			Simulation with measured microbial biomass C-to-N ratio and adjusted efficiency			
	Fraction	Characteristics	NiL	NPK	FYM	NiL	NPK	FYM	
Soil microbial Biomass	active	C-to-N ratio	6.5	6.5	9.0	4.7	5.3	4.8	
		Percentage of total	60	60	60	40	40	26	
		Decay rate (d <sup>-1</sup> )	15	5	5	10	10	10	
		Decay rate (d <sup>-1</sup> )	0.5	0.025	0.15	0.25	0.125	0.10	
Passive	Percentage of total	85	95	95	90	90	90	90	
	Decay rate (d <sup>-1</sup> )	0.02	0.01	0.015	0.01	0.005	0.005	0.005	
	Percentage of total	2.25	8	5	2	0.5	5	5	
	Decay rate (d <sup>-1</sup> )	0.02	0.003	0.008	0.04	0.03	0.008	0.008	
Soil organic matter	Resistant	Percentage of total	17.5	12	12.5	15	14.5	12.5	
		Decay rate (d <sup>-1</sup> )	0.0019	0.0005	0.0012	0.0019	0.0015	0.0006	0.0006
	Stable	Percentage of total	80.25	80	82.5	83	85	82.5	82.5
		Decay rate (d <sup>-1</sup> )	75 × 10 <sup>-8</sup>	75 × 10 <sup>-8</sup>	75 × 10 <sup>-8</sup>	75 × 10 <sup>-8</sup>	75 × 10 <sup>-8</sup>	75 × 10 <sup>-8</sup>	75 × 10 <sup>-8</sup>
Residual sum of squares			6211	7765	36,911	8704	14,126	58,396	

<sup>a</sup> The NIL plot was not fertilized, the NPK plot was annually fertilized with 144 inorganic N and the FYM plot with 35 t of farmyard manure for 151 y. The organic C content and total N content in the NIL plot was 8.7 g C kg<sup>-1</sup> dry soil (D.S.) and 1.01 g N kg<sup>-1</sup> D.S., 10.4 g C kg<sup>-1</sup> D.S. and 1.22 g N kg<sup>-1</sup> D.S. in the NIL plot and 30.2 g C kg<sup>-1</sup> D.S. and 2.96 g N kg<sup>-1</sup> D.S. in the FYM plot while the microbial biomass C and N were 146 mg C kg<sup>-1</sup> D.S. and 31 mg N kg<sup>-1</sup> D.S. in the NIL plot, 181 mg C kg<sup>-1</sup> D.S. and 37 mg N kg<sup>-1</sup> D.S. in the NPK plot and 502 mg C kg<sup>-1</sup> D.S. and 104 mg N kg<sup>-1</sup> D.S. in the FYM plot.

Table 2

Parameters obtained from an iterative curve fitting procedure of rye-amended soil from section 1 of the Broadbalk Continuous Wheat experiment at Rothamsted resulting in the lowest residual sum of squares and only considering dynamics of C, i.e. microbial biomass C and production of  $\text{CO}_2$  and an efficiency of 60<sup>a</sup>

Parameter	Characteristics	NIL	NPK	FYM
Rye active fraction	Decay rate ( $\text{d}^{-1}$ )	0.13	0.09	0.35
Rye resistant fraction	Decay rate ( $\text{d}^{-1}$ )	0.015	0.005	0.007
Rye stable fraction	Decay rate ( $\text{d}^{-1}$ )	$50 \times 10^{-6}$	$30 \times 10^{-6}$	$5 \times 10^{-6}$
Residual sum of squares		20,727	47,134	69,302

<sup>a</sup> The NIL plot was not fertilized, the NPK was annually fertilized with 144 inorganic N and the FYM plot with 35 t of farmyard manure for 151 yr. Of the total C added with the rye 63.0% belonged to the active fraction, 30.8% to the resistant and 6.2% to the stable fraction.

#### 4.3. Production of $\text{CO}_2$ in the rye-amended soil

Application of rye increased the  $\text{CO}_2$  production by 215, 246 and 284  $\text{mg C kg}^{-1}$ , in the NPK, NIL and FYM plot, respectively, compared with the unamended soil (Fig. 1b). The production of  $\text{CO}_2$  in the first 7 d of the incubation was comparable for each plot and differences between the plots were only noticeable towards the end of the incubation. If no priming effect was assumed then 44, 50 and 56% of the applied C was mineralized after 180 d, pointing at a possible effect of the plot from which the soil was sampled. The  $\text{CO}_2$  production was simulated satisfactorily (Fig. 1b) and the decay rate of the active fraction was larger for the FYM plot compared with the other plots (Table 2).

#### 4.4. Biomass C in the rye-amended soil

The microbial biomass C increased after the application of rye, on average with 59  $\text{mg biomass-C kg}^{-1}$  D.S. Simulated microbial biomass C values showed a sharp increase in the first day of the incubation followed by a smaller increase in the next days (Fig. 2b). A subsequent decrease started after approximately 15 d in the FYM plot and 40 d in the NIL and NPK plots.

#### 4.5. N dynamics in the unamended soil

The  $\text{NO}_3^-$  concentration increased almost linearly with production rates of 109, 111 and 750  $\mu\text{g N kg}^{-1} \text{d}^{-1}$  in the NIL, NPK and FYM plots, respectively (Fig. 3a). Increases in  $\text{NO}_3^-$  concentrations were not significantly different between the NIL and NPK plots ( $P < 0.05$ ) but were some 7.5 fold greater in the FYM plot.

We could not simulate the  $\text{NO}_3^-$  concentrations and microbial N satisfactorily if the C-to-N ratio as measured by the fumigation extraction method was used (Fig. 3a). The average microbial biomass C-to-N ratios measured were 4.7, 5.3 and 4.8 for the NIL, NPK and FYM, respectively (Table 3). If we used those values in the model then a permanent immobiliz-

ation of  $\text{NO}_3^-$  occurred within 7 d in the NIL and FYM plots while only a small increase in  $\text{NO}_3^-$  concentrations occurred in the NPK plot. The  $\text{NO}_3^-$  concentrations and the microbial biomass N in the NIL and NPK plot were satisfactorily simulated when C-

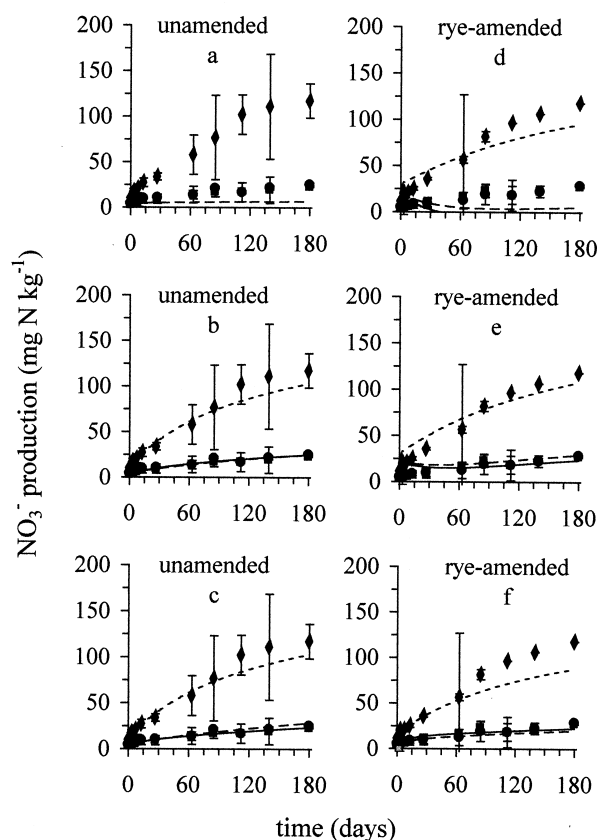


Fig. 3. Measured  $\text{NO}_3^-$  concentration ( $\text{mg N kg}^{-1}$  D.S.) in unamended soil and amended with 1022  $\text{mg rye kg}^{-1}$  D.S. from the unfertilized NIL plot (■), from the NPK plot annually fertilized with 144  $\text{kg N ha}^{-1}$  (●) and the FYM plot annually amended with farmyard manure (◆). Simulated values for the NIL (—), NPK (---) and FYM plot (---) assuming (a, d) an efficiency of 60 and measured microbial biomass C-to-N ratios, (b, e) an adjusted microbial biomass C-to-N and (c, f) an adjusted efficiency. Soil incubated aerobically for 180 d at 25°C. Bars indicate  $\pm 1$  standard deviation.

Table 3

Microbial biomass C-to-N ratio in unamended and rye-amended soil from section 1 of the Broadbalk Continuous Wheat experiment at Rothamsted incubated aerobically for 180 d at 25°C

Time (d)	Unamended soil <sup>a</sup>			Rye-amended soil <sup>a</sup>		
	NIL	NPK	FYM	NIL	NPK	FYM
0	4.2	3.6	4.0	5.5	4.9	4.1
1	4.3	4.0	5.0	4.6	5.6	5.5
3	4.9	4.5	4.8	7.9	6.8	5.3
7	5.5	6.3	5.7	5.2	6.9	5.9
13	3.2	6.3	5.4	3.9	5.3	4.9
27	ND	7.3	5.4	8.7	8.0	5.3
63	6.3	7.8	ND	7.9	7.0	ND
85	ND	ND	ND	5.9	6.4	ND
112	ND	4.6	ND	3.7	8.2	ND
140	ND	4.4	ND	3.5	4.0	ND
180	ND	ND	ND	ND	ND	ND
Mean	4.8	5.4	5.2	5.7	6.3	5.2

<sup>a</sup> ND = not determined.

to-N ratios of 6.5 and a C-to-N of 9 in the FYM plot were used (Figs. 3b and 4a) (Table 1).

There are different possible explanations for this phenomenon:

1. The C-to-N measurements were incorrect. This seems unlikely as the values were comparable with other measurements made on Broadbalk. Joergensen and Brookes (1990) reported a biomass C-to-N of 4.1 while Shen et al. (1989) reported values ranging from 4.3 to 6.3 depending on time of sampling and amount of inorganic fertilizer applied annually. Joergensen et al. (1992) reported an average C-to-N

ratio of 4.7 for arable soils.

- The microbial biomass we were measuring reflects only part of the total microbial biomass, i.e. the cytoplasmic constituents. Ladd et al. (1977) pointed out that the chloroform-labile biomass N may be more closely related to the cytoplasmic constituents of the soil biomass than to the cell wall N which has a greater C-to-N ratio. McGill et al. (1981) used in their model a C-to-N of 30 for structural and a C-to-N of 3 for metabolic biomass components.
- A part of the soil organic matter, e.g. the active fraction, had a C-to-N ratio that was much smaller than the C-to-N of the remaining soil organic matter. If we assumed the C-to-N ratio of the active fraction to be half of that of the soil organic matter as a whole (ca. 4.5) then we could simulate the N mineralization in the NPK plot but not in the NIL and FYM plot. Additionally, the only fractions of soil organic matter that are likely to have such a narrow C-to-N ratio are metabolites of soil micro-organisms. It is difficult to envisage that the amount of those metabolites was greater than the soil microbial biomass.
- The factor used to calculate the microbial biomass N (total N in the extract of the fumigated soil minus that in the unfumigated extract) was not 2.22 as reported by Jenkinson (1988) but lower, i.e. 1.62, 1.81 and 1.20 for the NIL, NPK and FYM plot, respectively to obtain a C-to-N ratio of 6.5 for the NPK and NIL plots and 9 for the FYM plot. Brookes et al. (1985) reported in the original method a conversion factor of 1.85: a value comparable with the one required to ensure a good fit for the NPK plot but not sufficiently large for the NIL and FYM plots.
- There is, however, another factor that affects the link between the C and N mineralization and the microbial biomass dynamics, i.e. the C efficiency. The efficiency indicates which amount of C is incorporated into the microbial biomass while the rest evolves as CO<sub>2</sub>. The model assumes a C efficiency of 60% (Payne, 1970); a factor that allowed us to simulate C and N mineralization in earlier experiments (Dendooven, loc cit.) considering a C-to-N ratio for the microbial biomass of 6 (Powlson and Jenkinson, 1976; Voroney and Paul, 1984). An efficiency of 60% was presumably too large and can be used for the decomposition of glucose (Nicolardot et al., 1994) but not for other organic material. The C and N dynamics in the NIL and NPK plot could be simulated satisfactorily (Figs. 1c, 2c, 3c and 4c) if we assumed an efficiency of 40 instead of 60%, i.e. less C is used for cell growth thus less inorganic N is assimilated and more N is mineralized. The decomposition rates were adjusted accordingly (Table 1). An efficiency of 40% has often been

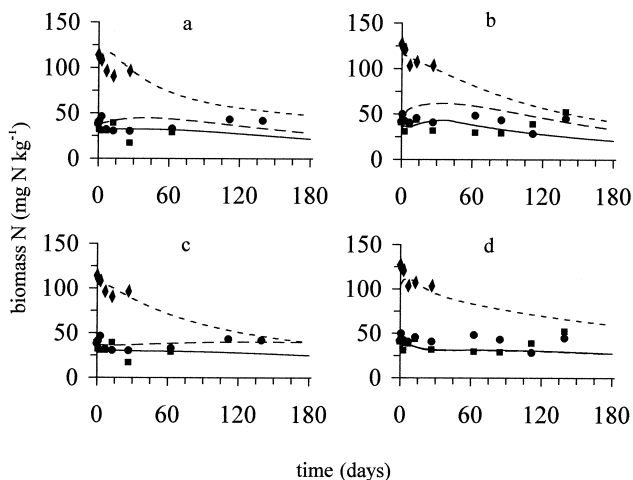


Fig. 4. Microbial biomass N (mg N kg<sup>-1</sup> D.S.) from the unfertilized NIL plot (■), the NPK plot annually fertilized with 144 kg N ha<sup>-1</sup> (●) and the FYM plot annually amended with 35 t farmyard manure ha<sup>-1</sup> (◆) and aerobically incubated for 180 d at 25°C. Legends to the simulated values can be found in Fig. 1. Bars indicate  $\pm 1$  standard deviation.

reported in literature (e.g. Rosswall, 1982) and used in C and N mineralization models (e.g. Parnas, 1975). The efficiency, however, had to fall to 26% in the FYM plot, a small value, to simulate the data satisfactorily.

If the difference between the FYM plot and the NPK and NIL plots was related to a difference in efficiency for C then that could be related to the composition of the C substrate available. van Veen et al. (1985) assumed that the efficiency was 60% for the active fraction, 40% for resistant and only 20% for the stable fraction. It is, however, difficult to indicate why the composition of organic material would be that different between the NIL and NPK plots and the FYM plot unless the application of farmyard manure affected the overall efficiency of C utilization. Another possibility is a difference in the dynamics of N between the FYM plot and NPK and NIL plots; i.e. more N remained in an easily decomposable fraction in the FYM plot than in the NIL and NPK plots.

6. It is also possible that the model is incorrect in some way. The model has been used on numerous occasions to simulate C and N dynamics in soil (Dendooven, loc cit.; Dendooven et al., 1994; Dendooven and Anderson, 1995) but that does not exclude possible limitations or errors in its concepts. For instance, the model assumes a constant microbial biomass C-to-N ratio which may be an oversimplification of reality. The microbial biomass C-to-N ratio in the NPK plot increased from 3.6 to a maximum value of 7.8 after 63 d before it decreased again to 4.4 after 140 d.

#### 4.6. N dynamics in the rye-amended soil

Surprisingly, the addition of 30 mg of organic N with rye had no significant effect on the  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  or

$\text{NH}_4^+$  concentrations in soil (Fig. 3d).  $\text{NO}_3^-$  production rates were 129, 119 and 764  $\mu\text{g N kg}^{-1} \text{d}^{-1}$  in the NIL, NPK and FYM plots, respectively. Possible losses of inorganic N through  $\text{NH}_3$  volatilization are unlikely as the pH of the soil was not high enough. Losses of N through dissimilatory reduction of  $\text{NO}_3^-$  or denitrification are most unlikely as the soil was incubated at 40% of WHC which should normally provide sufficient  $\text{O}_2$  to the microorganisms and no decreases in concentrations of  $\text{NO}_3^-$  were found which might have indicated anaerobicity.

As for the unamended soil, simulating the  $\text{NO}_3^-$  concentrations in the rye-amended soil using an efficiency of 60% and the measured microbial biomass C-to-N ratios, i.e. 5.7, 6.3 and 5.2 in the NIL, NPK and FYM plots, respectively, lead to permanent immobilization of N in the NIL and NPK plots (Fig. 5d). The mean microbial biomass C-to-N ratio was larger when rye was added in the NIL and NPK plot but not in the FYM plot. A C-to-N ratio of 8, 8 and 9.5 gave the best fit for the NIL, NPK and the FYM plot respectively (Fig. 5e, Table 4). However, the pattern of simulated values did not fit the measured  $\text{NO}_3^-$  concentrations. The simulated values showed a sharp increase in the first 7 d of the incubation corresponding with a flush in  $\text{CO}_2$  production. This was not observed in the measured values.

The efficiency for the decomposition of the rye was different when simulating the dynamics of C between the plots when we used an efficiency of 40% for the soil organic matter decomposition and the measured microbial biomass C-to-N ratio (Fig. 5f). It was 45% in the NIL and 60% in the NPK plot but we could not simulate the  $\text{NO}_3^-$  concentration satisfactorily in the rye amended FYM plot without a poor fit for  $\text{CO}_2$  production (Fig. 1d) and microbial biomass C and N (Figs. 2d and 4d, respectively) (Table 4). This pointed

Table 4

Parameters obtained from an iterative curve fitting procedure of rye-amended soil from section 1 of the Broadbalk Continuous Wheat experiment at Rothamsted resulting in the lowest residual sum of squares and considering dynamics of C and N, i.e. microbial biomass C and N and production of  $\text{CO}_2$  and inorganic N<sup>a</sup>

		Efficiency of 60 and variable biomass C-to-N ratio			Variable efficiency and measured biomass C-to-N ratio		
		NIL	NPK	FYM	NIL	NPK	FYM
Microbial biomass	C-to-N ratio	8	8	9.5	5.7	6.3	5.2
	Efficiency	60	60	60	45	60	40
Rye active fraction	Decay rate ( $\text{d}^{-1}$ )	0.12	0.03	0.32	0.08	0.12	0.11
Rye resistant fraction	Decay rate ( $\text{d}^{-1}$ )	0.0036	0.0085	0.0180	0.0050	0.0040	0.0005
Rye stable fraction	Decay rate ( $\text{d}^{-1}$ )	$5 \times 10^{-6}$	$5 \times 10^{-6}$	$5 \times 10^{-6}$	$25 \times 10^{-6}$	$5 \times 10^{-6}$	$5 \times 10^{-6}$
Residual sum of squares		141,506	56,612	74,567	33,494	114,713	154,042

<sup>a</sup> The NIL plot was not fertilized, the NPK was annually fertilized with 144 inorganic N and the FYM plot with 35 t of farmyard manure for 151 yr. Of the total C added with the rye 63.0% belonged to the active fraction, 30.8% to the resistant and 6.2% to the stable fraction.



again at a difference in the dynamics of C and N between the NPK and NIL plots and the FYM plot.

A possible priming effect was not included in the model as no  $^{14}\text{C}$ ,  $^{15}\text{N}$ -labelled organic material was added, although, it is possible that priming occurred in the rye amended soil (Brookes et al., 1990). The size of the priming effect and organic material decomposed could be different between plots explaining differences in efficiencies found but this is difficult to test without adding  $^{14}\text{C}$ ,  $^{15}\text{N}$ -labelled organic material. Additionally, the microbial biomass C-to-N ratio was assumed to be constant in the model but appeared to increase and decrease again in the NIL and NPK plots.

It was found that:

1. using an efficiency of 60% the C and N dynamics could be simulated when we assumed that the microbial biomass C-to-N ratio was greater than the measured values; 6.5, 6.5 and 9 in the NIL, NPK and FYM plots, respectively. The microbial biomass C-to-N ratio had to be increased to 8, 8 and 9.5 in the NIL, NPK and FYM plots amended with rye, respectively, but not all the N was located in the active fraction.
2. using an efficiency of 40% allowed us to simulate the C and N dynamics in the NPK and NIL plots when the measured biomass C-to-N ratios were used but we had to reduce the efficiency to 26% to simulate the C and N dynamics in the FYM plot. We could not simulate the C and N dynamics in soils added with rye assuming the same efficiency for rye in each soil.

We conclude that the dynamics of C and N as governed by the microbial biomass C and N were different for the FYM plot compared with the NPK and NIL plots. The difference, a result of long-term organic fertiliser application, could be due to a difference in efficiency for C, a difference in C-to-N ratio of the microbial biomass; a difference not reflected in the measured values, or a difference in the dynamics of N.

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