AN ABSTRACT OF THE THESIS OF

Aissatou Ndiaye for the degree of Master of Science in Soil Science presented on November 24, 1998. Title: IMPACT OF A RED CLOVER WINTER COVER CROP ON CARBON AND NITROGEN MINERALIZATION BY MICROORGANISMS IN SOIL AGGREGATES.

Abstract approved:

Peter J. Bottomley

Although legumes have been widely studied for their nitrogen-fixing ability, it is uncertain to what extent legume cover crops achieve their nitrogen-fixing potential under the climatic conditions encountered in western Oregon. Furthermore, it is unknown what factors control the proportions of legume cover crop N that are either sequestered into soil organic matter, or that contribute to the N requirements of the following summer crop. Soil was sampled in mid-September 1997, after harvest of a summer broccoli crop, from plots located at the North Willamettte Research and Extension Center, Aurora, Oregon. Soil was sampled from main plots that had been either winter cover cropped with red clover (LN_0 and LN_1) or fallowed during the winter period (FN_0 and FN_1), and specifically from sub-plots in which the following summer crop had received either zero (N_0) or an intermediate (N_1) rate of N fertilizer as urea. Levels of total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and readily mineralizable C and N were measured in both whole soil samples and in different aggregate-size classes (<0.25, 0.25-0.5

0.5-1.0, 1.0-2.0, and 2-5mm) prepared by dry sieving the soil. Aggregate size-class

distribution was not affected by the cover crop treatment. Although there was no significant effect of cover crop treatment on either TKN or TOC levels in whole soil samples, TOC levels were consistently higher in the small aggregate size-classes <1 mm of the fallow than the legume treatment. There was a significantly higher level of mineralizable C in the < 0.25 mm size class of the legume than the fallow treatment. There was a trend for the level of mineralizable N to be greater in soil from the legume than the fallow treatment. However, N fertilizer had a significant positive effect on the level of readily mineralizable N in both fallow and legume cover-cropped treatments, it had a negative effect on TKN levels among all aggregate-size classes. There were differences in the levels of mineralizable N measured among the aggregate-size classes, and immobilization of N between 20 and 40 days of incubation also differed among the aggregate-size classes.

IMPACT OF A RED CLOVER WINTER COVER CROP ON CARBON AND NITROGEN MINERALIZATION BY MICROORGANISMS IN SOIL AGGREGATES

By

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Chapter 1. INTRODUCTION TO THE THESIS

LEGUME COVER CROPS AND SOIL FERTILITY

Cover crops have been used for many years to preserve and enhance soil quality (Burket, 1998). When established and managed successfully, cover crops can reduce soil erosion, improve soil moisture holding capacity, and increase the availability of soil nutrients (Luna, 1994). Cover crop legumes have been widely studied, and their beneficial effects were referred to in Roman writings 2000 years ago. Sir Humphrey Davey wrote in 1813 that peas and beans seem well adapted to prepare the ground for wheat and suggested that legumes might derive nitrogen from the atmosphere. By the 1930s, the mechanisms by which legumes enhance soil N availability through N_2 fixation and subsequent mineralization of organic N by soil microorganisms had become reasonably well understood (Fred et al., 1932; Waksman, 1932). Indeed, as Smith et al. (1987) pointed out, it would be difficult to claim that interest in the use of leguminous cover crops is a novel concept.

Nevertheless, during the last 10 years there has been a tremendous renewal of interest on the part of both researchers and producers in this old practice. This can be related to concern over the effect of intensive agricultural practices on environmental quality, and to higher N fertilizer prices, and reduction in soil quality (Auld and Mahler, 1989).

Considerable interest has been shown in the use of winter cover crops for improving soils cropped to summer vegetables in Western Oregon (Burket et al., 1997). Winter cover crops have shown some potential to reduce soil bulk density, increase water infiltration properties, and change the distribution of soil aggregate-size classes (McVay et al., 1989; Miller and Dick, 1995a). There is little information about how winter cover crops might contribute to accretion of soil organic matter on the one hand, while their residues are mineralized in sufficient amounts to partially meet the N requirements of the subsequent summer crop.

Furthermore, while legume cover crops have the potential to fix atmospheric N_2, and to recover residual N fertilizer remaining after harvest of the summer crop (Kauffman, 1994), it is uncertain whether or not legumes can achieve this potential under the climatic conditions encountered in Western Oregon. Considerable uncertainty remains about how environmental factors affect the quantity of N fixed. The magnitude of the N contribution of legume cover crops to a succeeding crop has been reported to vary from 64 to 227 kg N ha^{-1} (Mitchell and Teel, 1977; McVay et al., 1989; Auld and Mahler, 1989; Ranells and Wagger, 1992). Furthermore, because cover crops are grown during the winter and early spring months under sub-optimal growth conditions, it is unclear what are the relative contributions of soil N and atmospheric N to the plant N budget. For example, the effectiveness of N_2 fixation at low temperature is poorly documented. Zhang et al., 1995 reported that the onset of N_2 fixation was increasingly delayed by exposure of plants to temperatures below 17°C. In the latter study, (impact of low temperatures in soybean on nodulation and N_2 fixation), it was found that a few days at low temperature had a major impact on the

time to onset of N_2 fixation and subsequent N accumulation and plant growth. The amount of N fixed also varies with the growth stage of the legume, which may reflect the dynamic balance between the availability of soil N to the plant and the activity of the root nodules (Heichel, 1987). Knowledge of the comparative benefits of N fertilizers and green manure crops is essential if growers are to weigh the cost effectiveness of both treatments (Kauffman, 1994).

Bottomley, (1992) pointed out that the majority of clover rhizobia recovered from Oregon soils tend to be sub-optimably effective N_2 fixers on commercial cultivars. It is unclear to what extent this observation might impact the amount and quality of the cover crop residues.

NITROGEN IN AGRICULTURAL SOILS

Nitrogen is the mineral nutrient most often in short supply in soil for plant nutrition, and the fourth most common element in plant composition, being outranked only by C, H, and O (Paul and Clark, 1996). The biogeochemical cycling of N in ecosystems can be divided into an external and an internal cycle (Hart et al., 1994). The external cycle includes those processes that add or remove N from ecosystems, such as N_2 fixation, N leaching, runoff, and denitrification. The internal cycle consists of processes that convert N from one chemical form to another, or transfer N between ecosystem pools. These processes include N mineralization, microbial immobilization of N, and nitrification. Soils differ considerably in the amount of organic N that can be mineralized. Burket (1998) and Hassink (1994) reported that soil management affects organic N dynamics in agricultural soil. In the context of cover crops, it is not always

clear what the impact will be on N mineralization by soil. For example, while it is generally thought that legume residues decompose rapidly, other studies have indicated that legume N can be relatively recalcitrant if the polyphenol content is high (Palm and Sanchez, 1991). Furthermore, Mendes et al. 1999 (in press) showed that TKN accumulated to a greater extent in some aggregate-size classes from the red clover cover crop treatment than in other classes, implying that locations exist in soil structure where legume N might be physically protected from mineralization.

SOIL AGGREGATION

Soil aggregates are an agglomeration of sand, silt, clay and organic matter and are described as having diameters ranging between 0.25 and 10mm (Burns and Davies, 1986). Macroaggregates are usually defined as aggregates greater than 250 μm in diameter while, microaggregates range between 50 and 250μm diameter. The building blocks of soil aggregates are mineral particles of sand (> 50 μm), silt (2.5-50 μm), clay (< 2 μm), organic polymers, and living organisms (roots, fungi, bacteria). Ionic bonds (multivalent cations bridges such as Ca^{2+}, Al^{3+}, Fe^{3+}) mediate both particle-particle bonding and also organic-particle bonding. In addition to these building blocks, chemical reactions such as precipitation and/or flocculation may create aggregates. Furthermore, faunal activity, for example burrowing, digging, and ingestion of the soil (earthworms) also may facilitate aggregate formation. Plant roots and fungal hyphae play roles in aggregate formation as was discussed earlier by Tisdal and Oades (1982) and Gupta and Germida (1988). There are several types of aggregates:

- transient aggregates which may not last very long because the transient binding agents are rapidly decomposed by microorganisms. Polysaccharides are thought to be the most important group of transient binding agents. They include polysaccharides produced when various organic materials are added to soil and some of the polysaccharides associated with roots and the microbial biomass (Tisdall and Oades, 1982).
- Temporary aggregates, are those held together by roots and hyphae. They may persist for months or perhaps years and are affected by soil management (Tisdall and Oades, 1979).
- Persistent aggregates formed by humic materials associated with amorphous iron, aluminum oxides, and alumino-silicates.

According to Tisdall and Oades (1982), organic matter stabilizes aggregates mainly by forming and strengthening bonds between domains and between quartz particle. Jastrow and Miller (1991) discussed the relationship between soil organisms and soil aggregates. Lynch and Bragg (1985) pointed out that biotic factors play an important role in the stabilization of soil aggregates. Soil type needs to be considered when discussing soil fabric and biotic factors, because the effects of both biotic and abiotic factors on the soil structure are closely related to the soil being considered. Even though the soil biota influences the development and stabilization of soil structure, at the same time it is affected by the microenvironment created by aggregates (Bandick, 1997). Aggregates protect microorganisms (especially bacteria) from predation. The distribution of microorganisms and their activities in soil aggregates separated by wet

and dry sieving techniques have been studied by Beauchamp, (1988) and Singh and Singh, (1995)

Many studies have shown that cultivation has a negative effect on soil structure and that macroaggregates are more negatively affected than microaggregates (Gupta and Germida, 1988; Tisdall and Oades, 1982). Cambardella and Elliot, (1993) mentioned that aggregate stability and macroaggregate structure are greater in no-till systems than in plowed or subtilled soil. Angers et al., (1993b) reported that tillage practices have a negative effect on aggregate stability, and Chaney and Swift (1984) mentioned a distinct relationship between the organic matter content of a soil and the degree of aggregate stability. Good soil structure for crop growth depends on the presence of aggregates 1-10 mm diameter which remain stable when wetted (Tisdall and Oades, 1982). Stability of aggregates is principally influenced by rainwater and irrigation (water stable aggregates), and compaction by agricultural machinery (Burns, and Davies, 1986). When unstable aggregates are wetted, they disintegrate because they are not strong enough to withstand the pressure of entrapped air and unequal swelling. Emerson, 1967, called this process slaking. Slaking reduces the amount of soil in the large macroaggregates size class to near zero in cultivated soils (Cambardella, and Elliot, 1993). Seasonal effects on aggregate stability were discussed by Perfect et al., (1990). They reported that structural stability decreased during the summer in legume, grass, and corn cropping systems.

Aggregates influence the volume and size of pore space, and thereby play a role in gas movement, water- holding capacity, and water infiltration. Aggregates provide physical protection of soil organic matter. In an earlier study, Mendes et al. (1999) (in

press) showed that TKN levels differed among aggregate-size classes of the red clover winter cover crop treatment at North Willamette Research and Extension Center, Aurora, OR, and that the levels of net mineralizable N also differed among aggregate-size classes. We were interested in confirming if legume N was sequestered differentially among certain sizes of aggregates, and if the lability of the material might vary. Past history of the site shows that the yield of the legume cover crop is low relative to the demands of the summer crop for N. In this current study, therefore, we were also interested in examining the interaction between the legume cover crop and the N fertilizer applied to the summer crop on the soil microbial and chemical properties measured.

Chapter 2. IMPACT OF A RED CLOVER WINTER COVER CROP ON CARBON AND NITROGEN MINERALIZATION BY MICROORGANISMS IN SOIL AGGREGATES

INTRODUCTION

Cover crop legumes and their beneficial effects on soil fertility have been widely studied. By the 1930s, the mechanisms by which legumes enhance soil N availability through N_2 fixation and subsequent mineralization of organic N had become reasonably well understood. Considerable interest has been shown in the use of winter cover crops for improving soils cropped to summer vegetables in western Oregon (Burket et al., 1997). When established and managed successfully, cover crops can reduce soil erosion, increase the availability of soil nutrients, and improve water holding capacity (Luna, 1994). Cover crop legumes have the potential to fix atmospheric N_2, and recover residual N fertilizer remaining after harvest of the summer crop (Kauffman, 1994). However, it is uncertain whether or not legumes can achieve this potential under the climatic conditions encountered in western Oregon. Furthermore there is considerable uncertainty about how environmental factors affect the quantity of N fixed. In addition there is little information about how winter cover crops might contribute to accretion of soil organic matter, while their residues are mineralized in sufficient amounts to partially meet the N requirements of the subsequent summer crop. Further studies are needed to determine the potential of winter cover crop residues to build up soil organic matter, and to provide nutrients for the subsequent summer crop.

Many studies have shown that the activities and repartition of soil organisms differ among aggregate-size classes (Gupta and Germida, 1988; Beauchamp and Seech,

1990; Mendes and al., 1999 [in press]). In this current study, I was interested in confirming if legume N was recovered heterogeneously among aggregates-size classes, and if there was a variation in the mineralizability of the N. Furthermore, I was interested in examining the interaction between the legume cover crop and N fertilizer applied to the summer crop on the soil properties measured.

MATERIALS AND METHODS

Site description

Soil samples were collected from the vegetable crop rotation experiment initiated in 1989 at the North Willamette Research and Extension Center (NWREC), Aurora, Oregon. The soil is a Willamette silt loam (Pachic Ultic Argixeroll), and the site characteristics have been described elsewhere (Brandi-Dorn et al., 1997; Burket et al., 1997). Prior to the study being initiated, the site had been in a wheat-fallow for many years. Field treatments include three winter cover crop treatments in a vegetable crop rotation that alternates two summer crops, sweet corn (*Zea mays* L. cv. Jubilee) and broccoli (*Brassica oleracea* L. var italica cv. Gem) (Table 2.1). The three winter cover crop treatments were: (1) winter fallow (no cover crop), (2) red clover (*Trifolium pratense* L. cv. Kenland), and (3) triticale (X *Triticosecale Wittmack* cv.Celia). The cover crops were established by the "relay" method in which they are seeded (85 kg ha^{-1} for triticale, and 25 kg ha^{-1} for red clover) under the summer crop in late July to take advantage of irrigation. The strategy behind the relay procedure was to have the cover crop established prior to the onset of fall rains. Each year cover crops were incorporated into the soil by rototilling in mid-April, followed by seedbed preparation in mid-May, and the summer crop of sweet corn, or broccoli planted mid to late May. Weed control involved preplant applications of 2.24 kg ha^{-1} EPTC, (S-ethyldipropylthiocarbamate) for sweet corn. No herbicide was used for broccoli.

Table 2.1 Crop rotation at North Willamette Research and Extension Center (NWERC).

		Relay cover crop	
Year	Season	Fallow	Legume
1989	Fall	Fallow	Red clover
1990	Spring	Corn	Corn
	Fall	Fallow	Red clover
1991	Spring	Broccoli	Broccoli
	Fall	Wheat	Red clover
1992	Spring	Wheat	Corn
	Fall	Fallow	Red clover
1993	Spring	Broccoli	Broccoli
	Fall	Fallow	Red clover
1994	Spring	Corn	Corn
	Fall	Fallow	Red clover
1995	Spring	Broccoli	Broccoli
	Fall	Fallow	Red clover
1996	Spring	Corn	Corn
	Fall	Fallow	Red clover
1997	Spring	Broccoli	Broccoli
	Fall	Fallow	Red clover

Experimental design

The experimental design was a randomized complete block split-plot with four replications. The plots were 18m x 9m, with winter cover crop treatments as the main plots and N fertilizer rate as the subplots. The main plots were divided into three equal sub-plot areas of 54 m^2. Nitrogen fertilizer (urea) rates were: zero (N_0), medium (56 and 140 kg of N ha^{-1} for sweet corn and broccoli, respectively) (N_1), and the rate (N_2) recommended by Oregon State University Extension Service for commercial production (224 and 280 kg of N ha^{-1} for sweet corn and broccoli, respectively).

Soil sampling

In mid-September 1997, after the broccoli harvest, soil samples were collected from each of four replicate plots of the four treatments (fallow, legume, N_0, and N_1) with a shovel to a depth of 15 to 20 cm. Samples were not taken from N_2 treatment. A composite sample of soil was prepared from each replicate plot and placed in sealed plastic bags. A total of 16 samples were taken from the field and brought to the laboratory for analysis.

Soil sieving

In the laboratory, soil samples were gently crushed by hand and dried in the cold room (4°C) until they reached a moisture content ≤ 14% (wt/wt). To prepare the soil aggregates for experimentation, 200 g portions of soil were placed in the top of a nest of sieves on a Tyler Ro-Tap testing sieve shaker. The shaker operates at one speed producing 200 – 250 oscillations min^{-1}. Soil was sieved for three minutes into the

following aggregate size classes: <0.25, 0.25 to 0.5, 0.5 to 1.0, 1.0 to 2.0, and 2.0 to 5.0mm. Soil placed in the 5 mm sieve was considered to be the whole soil. The aggregates retained on each sieve were weighed and stored in plastic bags in the cold room (4°C) until analyzed.

Laboratory incubations

Twenty g portions of soil aggregates (or whole soil) were placed in canning jars (500 ml volume) and rewet with deionized water to 27% (wt/wt). Two analytical replicates were used for each field treatment. The jars were incubated at 25°C and readily mineralizable C and N were determined after 10, 20, 30, and 40 days. Ammonium and nitrate levels in the whole soil and aggregate-size classes were measured prior to incubating the soil.

Net mineralizable N

NO_3^- and NH_4^+ levels were determined by extracting one g portions of soil aggregates in 10 ml of 1M KCl in small Erlenmeyer flasks (50 ml) for 30 minutes. The suspensions were centrifuged at 10,000 rpm for 15 minutes, and the supernatants stored in 5 ml plastic tubes and frozen until analysis. Nitrate was determined by mixing 0.5 ml of the KCl supernatant with 5 ml of Szechrome NAS solution (Polysciences, Wilmington, PA). Two analytical replicates were used for each soil sample. The Szechrome reagent was prepared by mixing 5 g of Szechrome NAS with a liter of a 1:1 mixture of H_2SO_4 and H_3PO_4. After 1h of incubation, the absorbance was measured spectrophotometrically at 570 nm. The amount of NO_3^- was determined from a

standard curve prepared with known concentrations of NO_3-N (0.5, 1.00, 2.00, 3.00, 4.00 μg NO_3-N /ml).

NH_4^+-N was determined by following the procedure of Solorzano (1969). One ml of the KCl supernatant was mixed with 2 ml of phenol solution (10 % in 95 % ethanol), 2 ml of 0.5 % (wt/vol) sodium nitroprusside, and 5 ml of a 2:3 vol/vol mixture of bleach and alkaline sodium citrate (100 g sodium citrate, 5 g NaOH and 500 ml of water). The samples were incubated for 1 h and the absorbance measured spectrophotometrically at 620 nm. The amount of NH_4^+-N was determined from a standard curve prepared with known concentrations of NH_4^+-N (0.5, 1.0, 2.0, 3.0, 4.0 μg/ml NH_4^+-N). Because the NH_4^+-N levels were below the limit of detection after 10 days of incubation readily mineralizable N levels were calculated as the levels of NO_3^--N at the sampling time minus the levels of NH_4^+-N and NO_3^--N at time zero.

Readily mineralizable C

Mineralizable C was determined by measuring the CO_2 trapped in 10 ml of 0.3 M KOH pipetted into scintillation vials and placed in the canning jars. At 10 day intervals, the vials were removed and the KOH back titrated against a 0.1M HCl solution with phenolphthalein as indicator. The jars were tightly sealed with silicon lubricant applied to the rims and the lids. After each sampling new vials containing KOH were placed in the jars. This procedure was repeated at 10 day intervals for 40 days. The amount of CO_2-C was determined from the difference between the control and the samples.

Total Organic Carbon (TOC)

Samples of soil aggregates and whole soil were ground with a mortar and pestle to pass a < 0.25 mm sieve. TOC was determined by combustion to CO_2 in a DC-80 Dohrmann Carbon analyzer equipped with an infrared detector. A small quantity of soil (≤ 5 mg) was used for each analysis, and two analytical replicates were used per aggregate sample. The amount of TOC was obtained by comparison with a 40 µl standard solution of potassium hydrogen phthalate containing 2000 ppm carbon.

Total Kjeldahl Nitrogen (TKN)

The procedure described by Bremner (1982) was used to determine the TKN. One g portions of soil aggregates (or whole soil) were digested with 1 g of Kjeldahl catalyst (a mixture of 100 g K_2SO_4, 10 g $CuSO_4.5H_2O$, and 1 g of Selenium) and 10 ml of concentrated H_2SO_4 for about 3 h until the suspension cleared and became light green in color. Ten ml of distilled water was added to each sample. Ammonium was determined by steam distilling 1 ml aliquots of alkaline digest for 5 minutes into 5 ml of boric acid indicator (H_3BO_3) and back titrating with standard 0.005 M H_2SO_4.

Statistical analysis

The SAS statistical package (SAS Institute, Cary, NC) was used to analyze the data. Since the different aggregate-size classes and incubation times were considered as repeated measures, a repeated measure analysis of variance was used to analyze the data. The covariance structure was determined using the Huynh-Feldt condition (HF) (sphericity test) in which both time and size must satisfy the Huynh-Feldt condition

(HF). If the HF condition was not satisfied (P-value > 0.05), then the repeated measures design can be modeled as a split plot. If the HF condition is satisfied (P-value < 0.05), an F-test adjusted with the Greenhouse-Geisser (G-G) epsilon was used. For the maximum level of mineralizable N (Nmax) and the TOC data, the HF condition was satisfied, therefore a one factor repeated measure analysis of variance (size being the repeated term) was used. For the mineralizable N and C (10 – 40 days) data, both time and size satisfied the HF condition, a two factors repeated measures analysis of variance (incubation time and size being the repeated terms) was used. The variable size has six levels: the five

aggregate-size classes, plus the whole soil). The variable incubation time has four levels (day 10, 20, 30, 40 d of incubation). A multivariate analysis of variance was used to analyze the aggregate-size distribution. For TKN and NH_4^+-N, and NO_3^--N levels prior to incubation, the HF condition was not satisfied, and the repeated measure design was modeled as a split plot. Sources of variation included cover crop treatment, nitrogen fertilizer, aggregate size, incubation time and their interactions. Main effects were separated using Tukey's Studentized Range Test (HSD) at $\alpha = 0.05$.

RESULTS

Size distribution of soil aggregates

The cover crop and N fertilizer treatments did not significantly affect the aggregate-size distribution of the soil at the sampling time (fall 1997). However, the interaction between cover crop and N fertilizer was significant for the size fractions 2.00 –5.00 mm (P = 0.03), 1.00–2.00 mm (P = 0.01), and <0.25 mm (P = 0.01). Soil from the legume plots that received N fertilizer (N_1) had a larger quantity of the larger size classes (1.00-2.00 mm and 2.00-5.00 mm) and a smaller quantity of the smaller size classes (<0.25 mm) than the legume plots receiving no N fertilizer. The size class 0.25 – 0.5 mm represented the smallest proportion of soil for all the treatment combinations, and the proportion of soil associated with a specific aggregate-size class generally increased with increasing aggregate size (Figure 2.1.).

Total Organic Carbon levels among soil aggregates

Although the cover crop treatment did not significantly influence levels of TOC in the whole soil, the smaller size classes (≤ 0.25 mm and 0.5-1.0 mm) from the fallow treatment contained significantly more TOC (P = 0.01) (Table 2.2) than the same size classes from the legume treatment (Table 2.3). Furthermore, even though the differences were not statistically significant, the TOC levels in the larger size classes (1.00–2.00 mm and 2.00–5.00 mm) were consistently lower in the legume treatment than in the fallow treatment (Table 2.3, and Figure 2.2). Although levels of TOC were

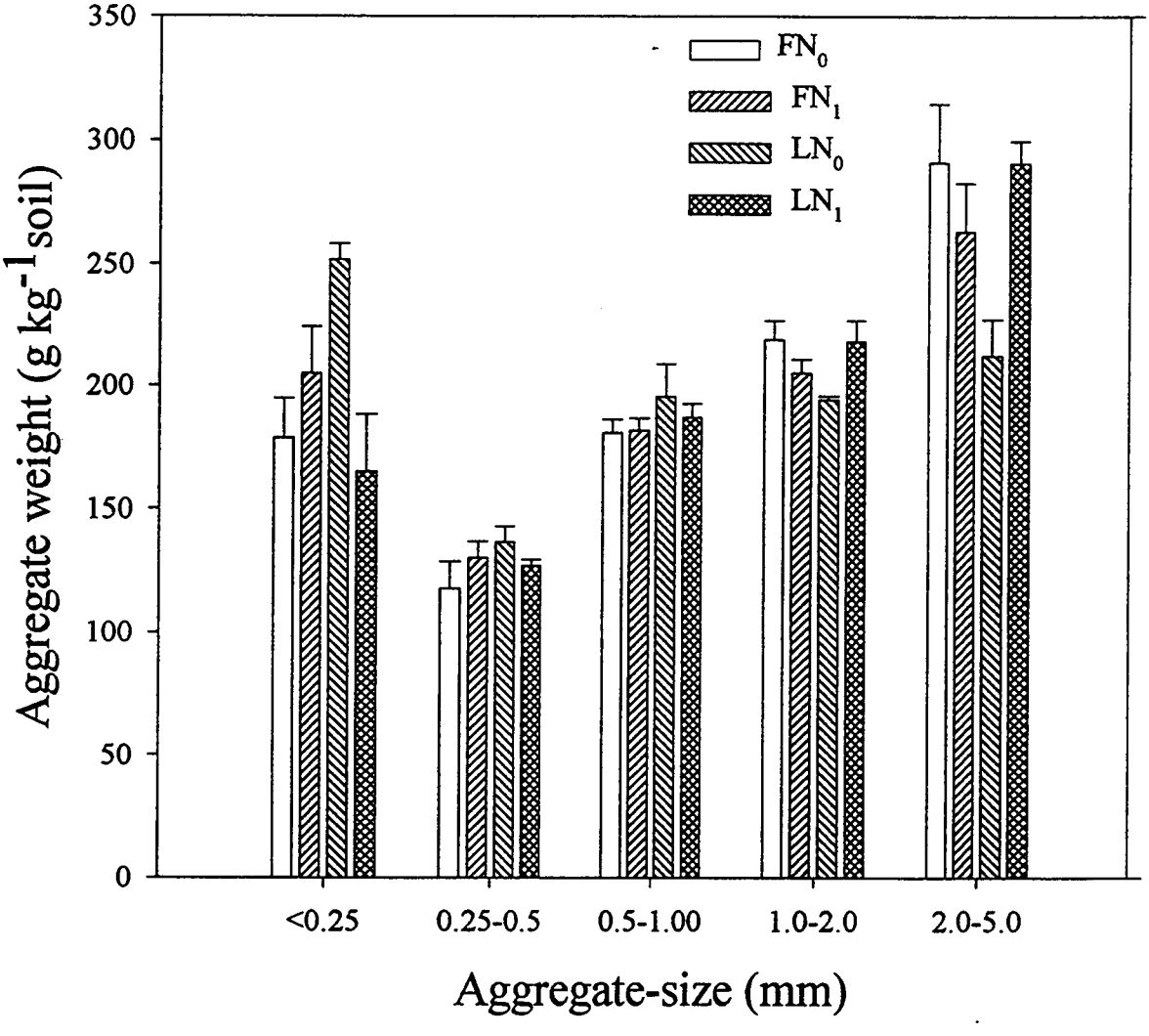

Figure 2.1. Cover crop and N fertilizer effects on the aggregate-size distribution.

Table 2.2 Summary of the repeated measure (Nmax, TOC) and split plot (TKN) analysis of variance for cover crop, nitrogen fertilizer, and aggregate-size effects.

Source of variation	Probability level (P>F)†		
	Nmax	TOC	TKN
Cover	0.02	0.0132	NS
Nitrogen	0.0001	NS	0.0001
Nitrogen × cover	0.036	NS	NS
Size	0.0001	NS	NS
Size × cover	NS	NS	NS
Size × Nitrogen	NS	NS	NS
Size × cover × Nitrogen	NS	NS	NS

† Abbreviations are: NS = not significant; Nmax = Maximum amount of readily mineralizable N
TOC = Total Organic Carbon

Table 2.3 Effect of cover crop and N fertilizer on TOC levels in the whole soil and among the different aggregate-size classes.

	Treatment			
	Cover crop		N fertilizer	
Aggregate size (mm)	Fallow	legume	N_0	N_1
<0.25	17.51 A‡	14.60 B	15.24 A	16.86 A
0.25 – 0.5	17.91 A	15.31 A	15.34 A	17.88 A
0.5 – 1.00	17.82 A	14.80 B	15.38 A	17.25 A
1.00 – 2.00	15.85 A	13.98 A	14.20 A	15.64 A
2.00 – 5.00	14.16 A	13.80 A	14.32 A	13.62 A
Whole soil	14.85 A	15.33 A	14.87 A	15.31 A

‡ Uppercase letters indicate comparisons made between either fallow and legume, or N_0 and N_1 for one aggregate-size class.
Values within each size fraction followed by the same uppercase letters are not significantly different at $P<0.05$.
Units are in g C kg^{-1} aggregate.

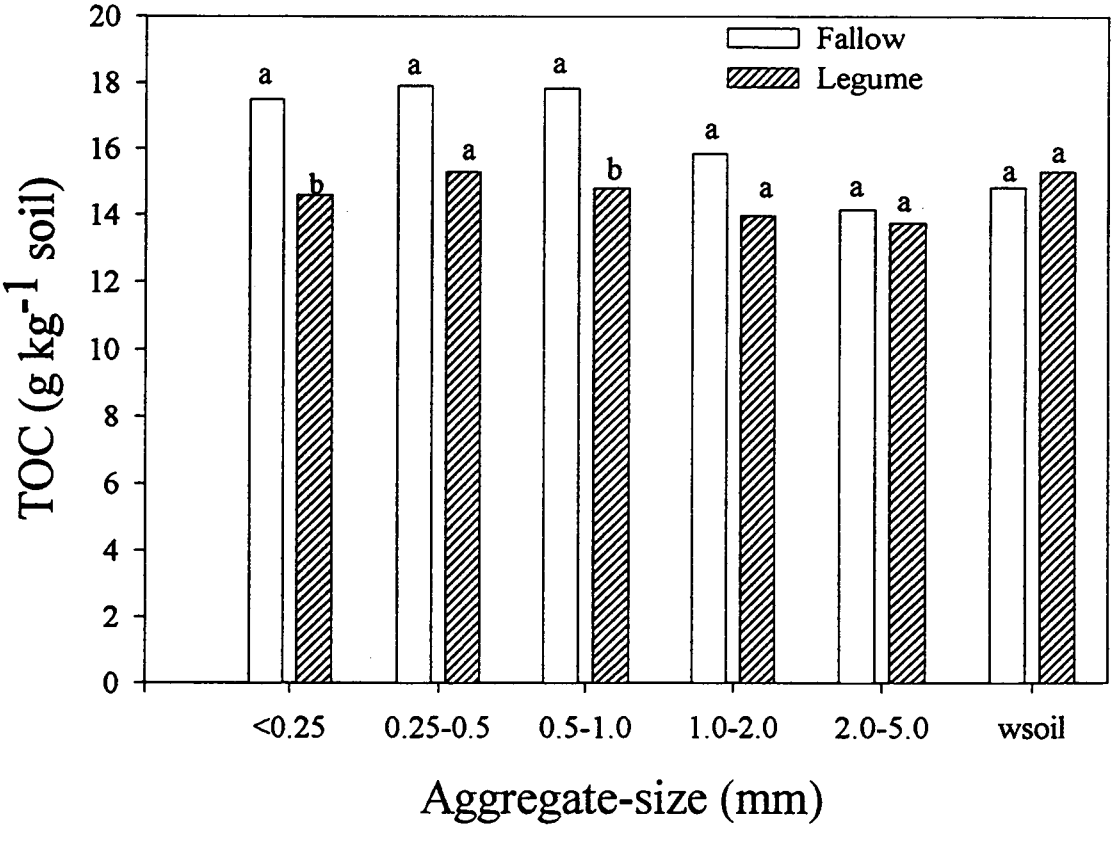

Figure 2.2. Effect of cover crop on TOC levels in whole soil and among the different aggregate-size classes. Values within the same size fraction followed by the same letters are not different at $P < 0.05$.

not significantly affected by fertilizer N, there was a trend for all aggregate-size classes < 2.00 mm from

the N fertilized plots to contain 1.4 to 2.5 g C kg^{-1} aggregate more TOC than their counterparts from N_0 treatment.

Total Kjeldahl Nitrogen levels among soil aggregates

Although the cover crop treatment did not significantly affect the levels of TKN, N fertilizer had a highly significant effect (P = 0.0001) on the TKN levels (Table 2.4). In contrast to the increase in TOC levels brought about by fertilizer N, TKN levels actually decreased in the N fertilizer treatment. There was approximately a 200 mg N kg^{-1} difference across all aggregate-size classes except for the 0.25-0.5 mm size class. TKN was not influenced by aggregate size, nor were there significant interactions between cover crop and N fertilizer, cover crop and aggregate size, and N fertilizer and aggregate size on TKN levels.

NH_4^+-N and NO_3^--N levels among aggregate-size classes before incubation.

Prior to incubation, NH_4^+-N levels were significantly greater in all aggregate-size classes obtained from the N fertilized soil than unfertilized soil (P = 0.0001) (Figure 2.3). The lowest NH_4^+-N level was found in the <0.25 mm size class regardless of N fertilizer. There were no significant differences detected between the amounts of NH_4^+-N in the fallow and in the legume treatment. However NH_4^+-N was slightly higher in the legume treatment.

Table 2.4 Effect of cover crop and N fertilizer on TKN levels in the whole soil and among the different aggregate-size classes.

	Treatment			
	Cover crop		N fertilizer	
Aggregate size (mm)	Fallow	Legume	N_0	N_1
<0.25	650 A‡	615 A	727 A	539 B
0.25 – 0.5	683 A	736 A	765 A	655 B
0.5 – 1.00	706 A	682 A	797 A	591 B
1.00 – 2.00	676 A	723 A	794 A	604 B
2.00 – 5.00	721 A	715 A	793 A	643 B
Whole soil	691 A	715 A	793 A	613 B

‡ Uppercase letters indicate comparisons made between either fallow and legume, or N_0 and N_1 for one aggregate-size class.
Values within each size fraction followed by the same uppercase letters are not significantly different at $P<0.05$.
Units are in mg N kg^{-1} aggregate.

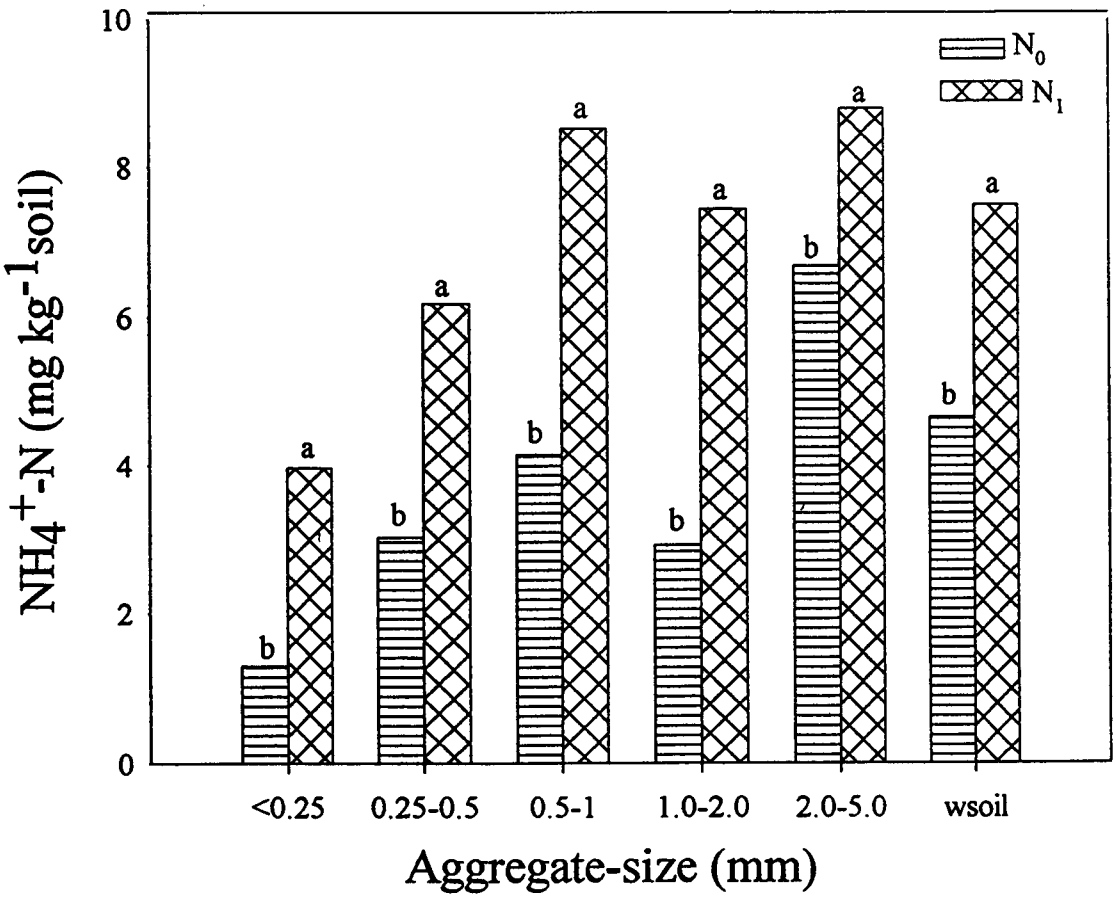

Figure 2.3. Effects of N fertilizer on NH_4^+-N levels prior to incubation of whole soil and the different aggregate-size classes.
Values within the same size fraction followed by the same letters are not different at P <0.05.

Prior to incubation the NO_3^--N levels were significantly influenced by the N fertilizer treatment (P = 0.0001) and by aggregate-size class (P = 0.0005). The NO_3^--N levels were significantly greater by 4 to 9 fold across all aggregate-size classes of the N fertilized treatments than from the unfertilized soil. Prior to incubation, the level of NO_3^--N in the fallow and in the legume treatment were not statistically different (Table 2.5) and (Figure 2.4).

Net mineralizable N

The level of net mineralizable N was significantly influenced by incubation time (P = 0.0001), but did not change as a function of cover crop, N fertilizer treatment (Table 2.6) or aggregate-size class. Even though the cover crop effect was not statistically significant, net mineralizable N levels were consistently higher in the legume treatment (Figure 2.5). Furthermore, after 10 and 20days of incubation more net mineralizable N was detected in the N_1 treatment than in the N_0 treatment (Figure 2.5). However, between 20 and 40 days of incubation, the amounts of mineralized N declined and this was especially apparent in the N_1 treatment. Regardless of the treatment or the aggregate-size class, the maximum level of mineralizable N was achieved at day 20. In the legume and the N_0 treatments, less immobilization of nitrate occurred between 20 and 30 days incubation than in fallow and N_1 treatments respectively (Figure 2.5). Between day 20 and day 30 incubation 3.87 mg NO_3^--N was immobilized in the fallow vs 3.22 in the legume treatment and 1.78 mg NO_3^--N in the N_0 vs 5.31 in the N_1 treatment.

Table 2.5 Effect of cover crop and N fertilizer on NO_3^--N levels prior to incubation of whole soil and the different aggregate-size classes.

	Treatment			
	Cover crop		N fertilizer	
Aggregate size (mm)	Fallow	legume	N_0	N_1
<0.25	8.03 A‡	8.02 A	3.72 B	12.33 A
0.25 – 0.5	4.39 A	4.67 A	0.92 B	8.14 A
0.5 – 1.00	7.13 A	6.13 A	1.42 B	11.84 A
1.00 – 2.00	7.18 A	7.15 A	1.98 B	12.35 A
2.00 – 5.00	7.79 A	6.45 A	2.58 B	11.66 A
Whole soil	7.01 A	7.48 A	1.49 B	13.00 A

‡ Uppercase letters indicate comparisons made between either Fallow and legume, or N_0 and N_1 for one aggregate size class.
Values within each size fraction followed by the same uppercase letters are not significantly different at $P<0.05$.
Units are in mg NO_3^--N kg^{-1} aggregate.

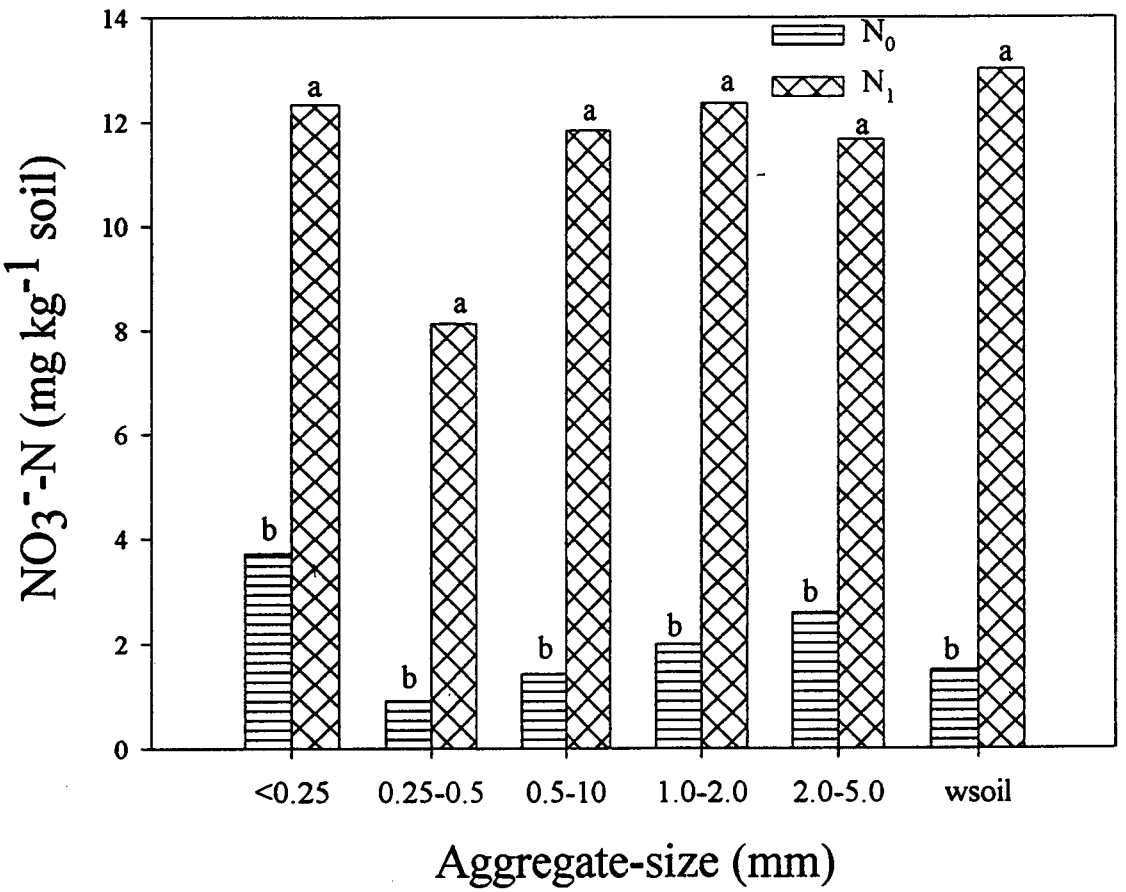

Figure 2.4. Effects of N fertilizer on NO_3^--N levels prior to incubation of whole soil and the different aggregate-size classes.
Values within the same size fraction followed by the same letters are not different at $P < 0.05$.

Table 2.6 Summary of the two factors repeated measures analysis of variance for cover crop, nitrogen fertilizer, aggregate-size and sampling time effects.

Source of variation	Probability level (P>F)†	
	NO_3^--N	CO_2-C
Cover	NS	NS
Nitrogen	NS	NS
Nitrogen × cover	NS	NS
Time	0.0001	0.04
Time × cover	NS	NS
Time × Nitrogen	NS	NS
Time × cover × Nitrogen	NS	NS
Size	NS	NS
Size × cover	NS	NS
Size × Nitrogen	NS	NS
Size × cover × Nitrogen	NS	NS
Time × Size	0.0002	0.0001
Time × Size ×cover	NS	0.05
Time × Size × Nitrogen	NS	NS
Time × Size × cover × Nitrogen	NS	NS

† Abbreviations are: NS = not significant; NO_3^--N = readily mineralizable N
CO_2-C = readily mineralizable C

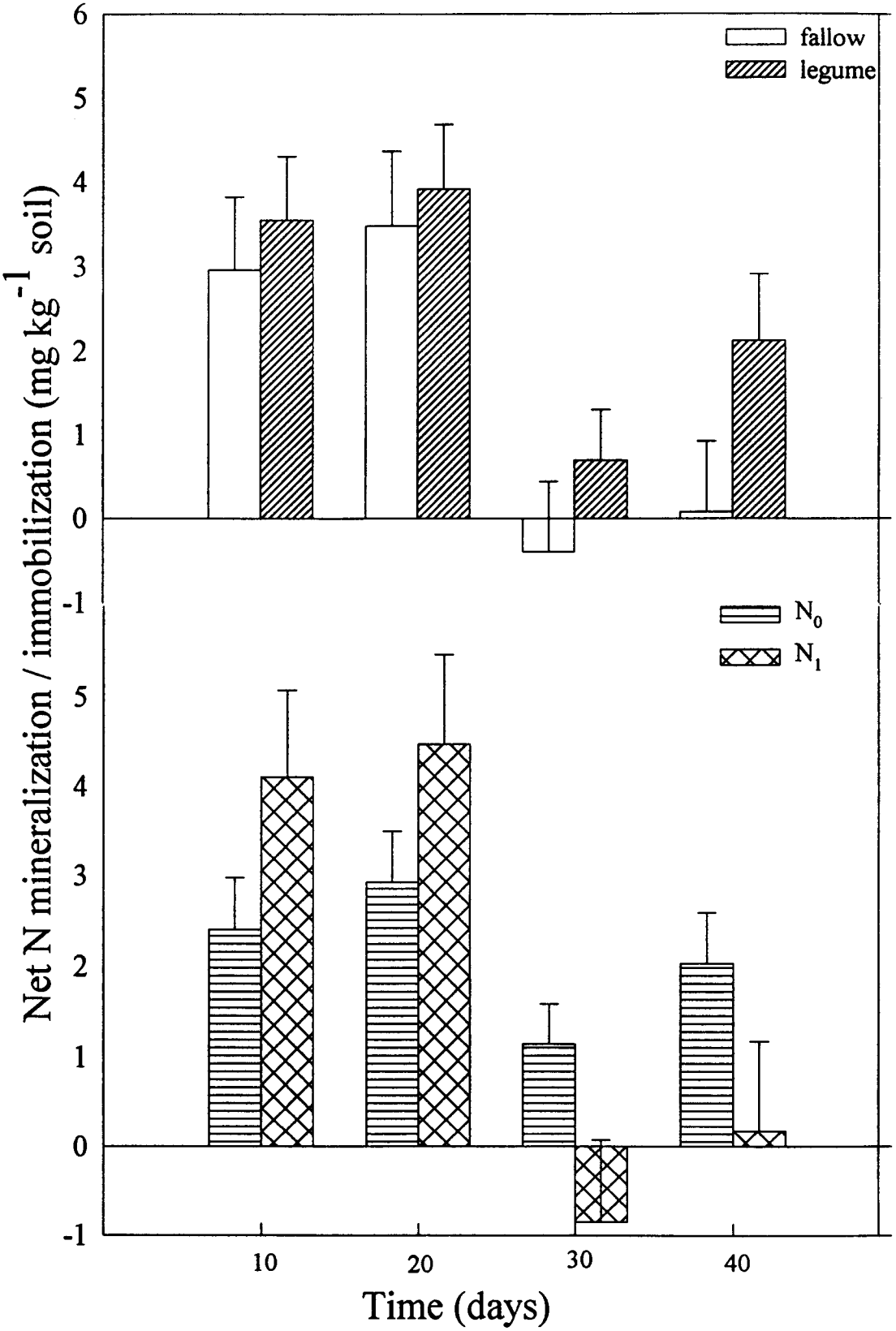

Figure 2.5. Cover crop and N fertilizer effects on the net N mineralization / immobilization.

Maximum level of net mineralizable N (Nmax)

The maximum level of net mineralizable N was significantly greater in the whole soil from the legume than the fallow treatment (5.8 vs. 2.4 mg NO_3^--N kg^{-1} soil aggregate) (Table 2.7). Furthermore, there were significant effects of aggregate size ($P = 0.0001$) and N fertilizer ($P = 0.0001$) on Nmax (Figure 2.6) It can be seen that the level of Nmax was much lower in the 2.00-5.00 mm size class than in other sizes. The maximum level of net mineralizable N was significantly greater in the 0.25-0.5 mm and the 1.0-2.0 mm aggregate-size classes of the N fertilized treatment than the N_0 treatment.

Readily mineralizable carbon

Neither cover crop, nor N fertilizer treatment affected the level of readily mineralizable C at the time of sampling. In contrast, the effect of incubation time ($P < 0.05$) was statistically significant (Table 2.3). Even though the main effect of aggregate-size class was not significant, the level of readily mineralizable C was also significantly influenced by the interaction between time and aggregate-size class. For example, in the size class <0.25 mm, the amount of mineralizable C produced was significantly greater in the legume treatment than in the fallow treatment after day 10 incubation (Table 2.8). Even though the difference was not significant, the same trend was observed for the aggregate-size class 2.00-5.00 mm. In contrast, an opposite trend was measured in the whole soil. At day 10, a higher level of readily mineralizable C was produced in the fallow than in the cover crop treatment. However the differences were not statistically significant.

Table 2.7 Effect of cover crop and N fertilizer on the maximum level of net mineralizable N in the whole soil and among the different aggregate-size classes.

	Treatment			
	Cover crop		N fertilizer	
Aggregate size (mm)	Fallow	legume	N_0	N_1
<0.25	4.25 A‡	6.81 A	4.26A	6.80 A
0.25 – 0.5	11.15 A	12.7 A	8.78 B	15.05A
0.5 – 1.00	5.80 A	4.83 A	4.60 A	6.02 A
1.00 – 2.00	8.60 A	6.83 A	5.50 B	9.91 A
2.00 – 5.00	0.50 A	1.56 A	0.90 A	1.97 A
Whole soil	2.40 A	5.80 B	3.20 A	5.00 A

‡ Uppercase letters indicate comparisons made between either fallow and legume, or N_0 and N_1 for one aggregate size class.
Values within each size fraction followed by the same uppercase letters are not significantly different at $P<0.05$.
Units are in mg NO_3^--N kg^{-1} aggregate.

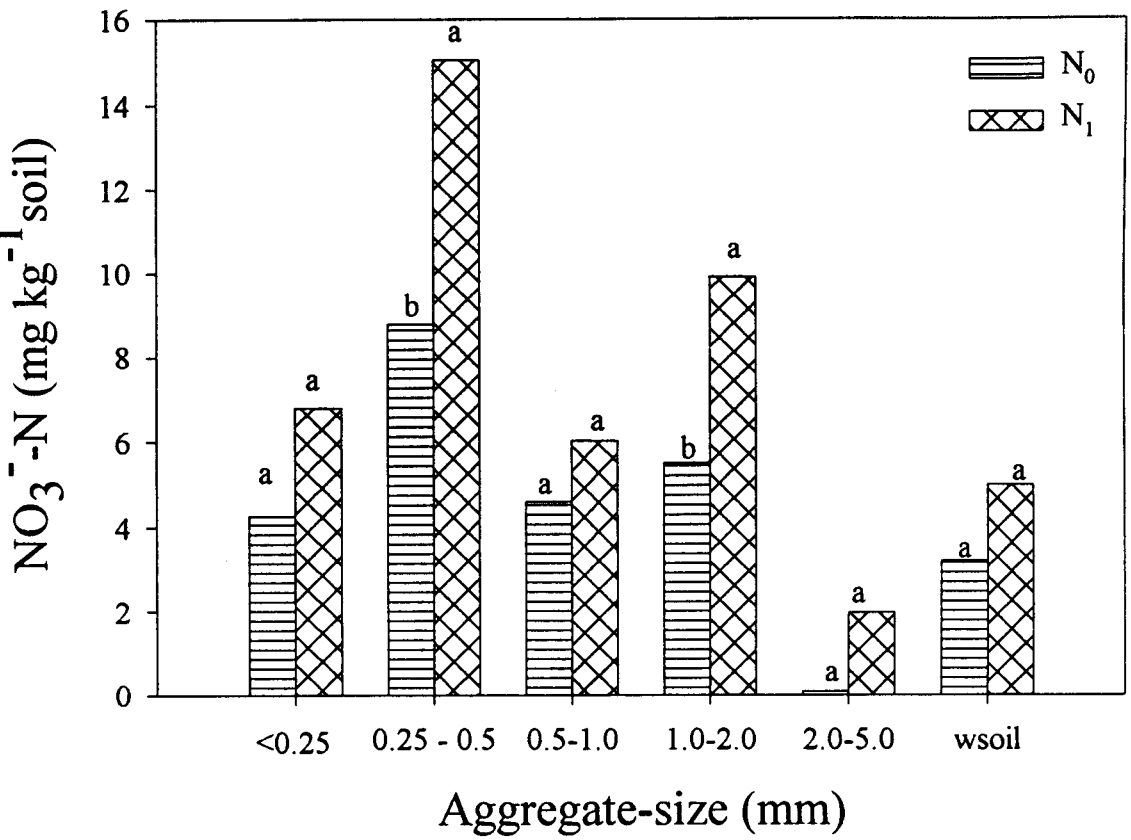

Figure 2.6. Effects of N fertilizer on the maximum level of net mineralizable N in the whole soil and among the different aggregate-size classes. Values within the same size fraction followed by the same letters are not different at $P<0.05$.

Table 2.8 Effect of cover crop and incubation time on the cumulative readily mineralizable C on the whole soil and among the different aggregate-size classes.

	Aggregate size classes (mm)					
	<0.25		2.00-5.00		Whole soil	
Time (days)	Fallow	Legume	Fallow	Legume	Fallow	Legume
10	89.00 A‡	109.81 A	94.60 A	103.40A	104.06A	122.20A
20	223.40 B	292.70 A	261.60A	271.97A	270.10A	202.90A
30	270.90 B	349.07 A	311.40A	321.82A	321.25A	256.65A
40	337.00 B	421.35 A	371.43A	399.80A	384.50A	318.73A

‡ Uppercase letters indicate comparisons made between fallow and legume for one aggregate size class within a sampling time.
Values followed by the same uppercase letters are not significantly different at $P<0.05$.
Units are in mg CO_2-C kg^{-1} aggregate.

was produced in the fallow than in the cover crop treatment. However the differences were not statistically significant.

DISCUSSION

Microbial activities that mediate the processes of organic matter turnover and nutrient cycling are regulated by agronomic practices like cultivation, residue management, and fertilization (Ajwa et al., 1998).

The failure of TOC and TKN levels from the red clover cover crop treatment to have shown any increase may be simply explained by the failure of the red clover cover crop to thrive under the relay establishment and to grow well during fall, winter and spring conditions. Cover crop yield data obtained from NWREC show that red clover yield is highly variable across the different plots ranging from 200 to 850 kg ha^{-1} dry matter in 1997, and quite variable from year to year (~ 2000 kg ha^{-1} in 1998) (Appendix 1, Table 1). A variety of factors could contribute to the low yield: yield could be poor due to lack of effective rhizobia, soil acidity, low temperatures and short day lengths. Further research work is required to discriminate among these possibilities to determine which have major causes.

Another possible explanation why TOC and TKN levels have not increase may be attributed to legume residues being mineralized rapidly and not protected in the soil structure, or to loss of residue N via volatilization. Janzen and McGinn (1991) reported that the volatile loss of labile N during the decomposition of legume residues might appreciably reduce its fertility benefit. They also pointed out that since N input from N_2 fixation is one of the most important benefits, potential loss of N from legume material is of considerable concern and merits quantification.

Not only was there no increase of TOC and TKN in soil from the red clover plots, there was actually a decrease in TOC in certain aggregate-size classes indicating

that growth of red clover and /or incorporation of residues enhanced mineralization of soil carbon from some locations. There are two possible reasons for this. First, the plots containing the cover crop are cultivated twice in the spring; once to incorporate residues, and secondly to prepare a seed bed for the summer crop. It is possible that the "extra" cultivation stimulates mineralization of TOC. Ajwa et al. (1998) pointed out that cultivation causes reduction in TOC, microbial biomass, and affects the activity of nitrification and denitrification enzymes. N'Dayegamiye et al. (1997) also reported that long-term mineral fertilizer applications coupled with crop rotations with low organic residue inputs could reduce organic matter levels in soil. They mentioned that long-term mineral fertilizer applications stimulate C mineralization and therefore are unfavorable to the accumulation of stable organic matter.

Another possible reason for lower TOC and TKN in the cover cropped plots might relate to the observation that mineralization of soil organic matter can be stimulated when N fertilizer is added to the soil and is referred to as the "priming" effect. Jenkinson et al. (1988) pointed out that the "added N interaction" can be accelerated by N fertilizer such as urea because the later can increase soil pH. More studies are required to determine if TOC mineralization is stimulated by incorporation of N-rich residues such as red clover and to determine if urea fertilizer plays a role.

My studies on the microbial properties of different aggregate-size classes provide additional information to earlier studies from the same soil (Mendes et al., 1999, in press). Ammonium and nitrate levels in the aggregates were influenced by fertilizer after harvest of the summer crop. In all aggregate-size classes, there was significantly more NH_4^+ in the fertilized than in the unfertilized plots. The significant

interaction between aggregate-size class and cover crop on the time zero NH_4^+ could indicate that ammonium does not penetrate equally into all size fractions, or that the properties of ammonium production from urea and the properties of nitrification differ among the size classes.

Not only was there a difference in the maximum level of mineralizable N among aggregates, there were different immobilization characteristics. More mineralizable N was released from the 0.25-0.5 mm and 1.00-2.00 mm N_1 treatment implying that these sizes had sequestered N and prevented its mineralization until the N was exposed during the aggregate-size separation. Other researchers have also shown that N mineralization differed among the aggregate-size classes. Gupta and Germida (1988) pointed out that macroaggregates have a higher level of N mineralization than micro aggregates. Further studies are required to understand why the levels of mineralizable N differ among aggregate-size classes.

Our mineralizable C data illustrate that cover crop and aggregate size interact in the protection of mineralizable C. More mineralizable C was detected in the <0.25 mm of the legume than in the fallow treatments even though there were no cover crop effects on the whole soil. Presumably labile C in the <0.25 mm of legume treatment was protected in the whole soil state and did not influence whole soil respiration. However, it was observed that TOC levels were consistently higher in the small size fractions ≤ 1 mm of the fallow treatment, which is consistent with the increased labile C in the smaller aggregate-size of the red clover treatment.

While TOC and TKN levels showed no increase in the legume treatment, there was a trend for levels of readily mineralizable N to be larger in the legume cover

cropped soil as compared with the fallow plots. A more detailed comparative study of the levels of readily mineralizable C and N among all aggregate-sizes is required to determine why mineralizable C and N properties do not follow the same trends.

LITERATURE CITED

Ajwa, H. A., C. W. Rice, and D. Stomayor. 1998. Carbon and nitrogen mineralization in tallgrass prairie and agricultural soil profiles. Soil Sci. Soc. Am. J. 62: 942-951.

Angers, D. A., N. Samson, and A. Lègère. 1993. Early changes in water-stable aggregation induced by rotation and tillage in a soil under barley production. Can. J. Soil Sci. 73: 51-59.

Bandick, A. K. 1997. Field management effects on the thermal stability and activity of soil enzymes in whole soil and aggregates. MS. thesis, Department of Crop and Soil Science, Oregon State University, Corvallis, OR.

Beauchamp, E. G. and A.G. Seech. 1990. Denitrification with different size of soil aggregates obtained from dry-sieving and from sieving with water. Biol. Fertil. Soils. 10: 188-193.

Bottomley, P. J. 1992. Ecology of *Bradyrhiobium* and *Rhizobium*. In: Biological nitrogen fixation (Stacey, G. et al., eds.) pp. 292-347. Chapman and Hall, New York.

Brandi-Dohrn, F. M., R. P. Dick, M. Hess, S. M. Kauffman, J. D. D. Hemphill, and J. S. Selker. 1997. Nitrate leaching under a cereal rye cover crop. J. Environ. Qual. 26: 181-188.

Bremner, J. M. 1996. Nitrogen Total. pp. 1085-1121. In: D. L. Sparks (ed.) Methods of soil analysis. Chemical methods, 2nd edition. American Society of Agronomy, Madison, WI.

Burket, J. Z., 1998. Cover crops and biological functional diversity in relation to nitrogen in soil. Ph. D thesis, Department of Crop and Soil Science Oregon State University, Corvallis, OR.

Burket, J. Z., D.D. Hemphill and R.P. Dick.1997. Winter cover crops and N management in sweet corn and broccoli rotations. Hortscience 32:664-668.

Burns, G., and Davies, A. 1986. The microbiology of soil structure. Biol. Agric. Hort. 3: 95-113.

Cambardella, C. A. and E. T. Elliot. 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soil. Soil Sci. Soc. Am. J. 57:1071-76.

Chaney, K., and Swift, R. S. 1984. The influence of soil organic matter on aggregate stability in some British soils. J. Soil Sci. 35: 223-230.

Emerson, W. W. 1967. A classification of soil aggregates based on their coherence in water. Aust. J. Res. 5: 47-57.

Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. Root nodule bacteria and leguminous plants. Studies in Sciences n° 5. University of Wisconsin, Madison, WI.

Gupta, V.V.S.R., and Germida, J. J. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol. Biochem. 20: 777-786.

Hart, S.C., Stark, J.M., Davidson, E.A., and Firestone, M.K. 1994. Nitrogen mineralization, immobilization and nitrification. pp. 987-1016. In: R.W. Weaver, J. S. Angle, and P. J. Bottomley (eds). Methods of soil analysis. Microbiological and biochemical properties ASA, Madison, WI.

Hassink J. 1994. Effect of soil texture and grassland management on soil organic C and rates of C and N mineralization. Soil Biol. Biochem. 26: 1221-1231.

Heichel, G. H. 1987. Legumes as a source of nitrogen in conservation tillage systems. pp.29-34. In: J. F. Power (editor). The role of legumes in conservation tillage systems. Soil Conservation Society of America, Ankeny, Iowa.

Janzen, H. H., and S. M. McGinn. 1991. Volatile loss of nitrogen during decomposition of legume green manure. Soil Biol. Biochem. 23: 291-297.

Jastrow, J. D., and R. M. Miller. 1991. Methods for assessing the effects of biota on soil structure. Agri. Ecos. and Envir. 34: 279-303.

Jenkinson, D. S., R. H. Fox, and J. H. Rayner. 1988. Interactions between fertilizer nitrogen and soil nitrogen, the so-called priming effect. J. Soil Sci. 36: 425-444.

Kaufman, S. M. 1994. Inorganic N and soil biological dynamics in cover crop systems. MS. thesis, Department of Crop and Soil Science, Oregon State University Corvallis, OR.

Luna J. 1994. Multiple impacts of cover crops in horticultural systems. Proc. Ore. Hort. Soc. 84: 198-205.

Lynch, J. M. and E. Bragg. 1985. Microorganisms and soil stability. Adv. Soil Sci. 21: 133-177.

Mahler R. L. and Auld D. L. 1989. Evaluation of the green manure potential of Austrian winter peas in northern Idaho. Agron. J. 81: 258-64.

McVay, K. A., D. E. Radcliffe, and W. L. Hargrove. 1989. Winter legume effects on soil properties and nitrogen fertilizer requirements. Soil Sci. Soc. Am. J. 31: 1856-1862.

Mendes, I., A. K. Bandick, R. P. Dick, and P. J. Bottomley. 1999.In Press. Effects of winter cover crops on the distribution of microbial biomass and associated activities among soil aggregate-size classes (in press). Soil Sci. Soc. Am. J.

Miller, M., R. D. Dick. 1995. Dynamics of soil C and microbial biomass on whole soil aggregates in two cropping systems differing in C-input. Appl. Soil Ecol. 2: 253-261.

Mitchel W. H. and Teel M. R. 1977. Winter annual cover crops for no tillage corn production. Agron. J. 69: 569-73.

N'Dayegamiye, A., M. Goulet and M. R. Laverdière. 1997. Effet a long term d'apport d'engrais mineraux et de fumier sur les teneurs en C et N des fractions densimétriques et des aggrégats du loam limoneux Le bras. Can. J. Soil Sci. 77: 351-58.

Palm, C. A., and Sanchez, P. A. 1991. Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biol. Biochem. 23: 83-88.

Paul, E.A., and Clark, F. E. 1996. Soil microbiology and biochemistry. 2nd ed. Academic Press, San Diego, CA. 339pp.

Perfect, E., B. D. Kay, W. K. P. van Loon, R. W. Sheard, and T. Pojasok. 1990. Factors influencing soil structure stability within a growing season. Soil. Sci. Soc. Am. J. 54: 173-179.

Ranells, N. N., and M. G. Wagger. 1992. Nitrogen release from crimson clover in relation to plant growth stage and composition. Agron. J. 84: 424-430.

SAS Institute. 1995. Selected SAS® Documentation: Design and Analysis of experiments (fall, 1995). SAS Institute Inc, Cary, NC. 484pp.

Seech, A. G., and E. G. Beauchamp. 1988. Denitrification in soil aggregates of different sizes. Soil Sci. Soc. Am. J. 52: 1616-1621.

Singh, S., and J. S. Singh. 1995. Microbial biomass associated with water-stable aggregates in forest, savanna and cropland soils of a seasonally dry tropical region, India. Soil Biol. Biochem. 27: 1027-1033.

Smith M., W.W. Frye, J.J. Varco. 1987. Legume winter cover crops. Adv. Soil Sci. 7: 95-139.

Tisdall, J.M., and Oades, M. 1979. Stabilization of soil aggregates by the root system of ryegrass. Aust. J Soil Res.17: 509-514.

Tisdall, J. M., and Oades, M. 1982. Organic matter and water stable aggregates in soil. Soil Sci. Am. J., 33: 141-163.

Waksman, S. A. 1932. Principles of soil microbiology, 2nd ed. The Williams and Wilkins Company, Baltimore, MD.

Zhang, F., D. H. Lynch and, D. L. Smith. 1995. Impact of low temperature in soybean (Glycine max. L. Merr.) on nodulation and nitrogen fixation. Environ. Exper. Bot. 35: 279-85.

APPENDIX

Table A1. Red Clover cover crop yield: Biomass (dry matter kg ha^{-1}) and total N (kg ha^{-1}) across the 4 blocks.

	Yield			
	Biomass(dry matter kg ha^{-1})		Total N (kg ha^{-1})	
Block	N_0	N_1	N_0	N_1
1	573	747	18	18
2	499	592	15	13
3	360	198	10	4
4	585	846	14	21

Table A2. Broccoli yield: Biomass (dry matter kg ha^{-1}) across the 4 blocks.

	Yield Biomass (dry matter kg ha^{-1})			
Block	Fallow N_0	Fallow N_1	Legume N_0	LegumeN_1
1	48	150	152	82
2	85	92	90	111
3	31	96	30	81
4	82	115	111	111