Sequestering C in Stable Soil Organic Matter Fractions: How Important is Fertiizer-N in Sequestering C

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Summary

Many nutrient cycling models use the C-to-N ratio of residues as a proxy to predict decomposition rates and soil organic matter (SOM) formation. However, there are indications that added soil mineral-N as fertilizer or atmospheric deposition as an alternative N-source for microorganisms may affect the decomposition pathway and ultimately the formation of stable SOM. We investigated how the effect of mineral-N on the transformation of microbial biomass C and N is modified by residue quality, and whether residue-C and residue-N transformations remain linked upon mineral-N additions. In a 40-d incubation, the dilution of ^{13}C and ^{15}N in labeled microbial biomass was followed in each of the following combinations of mineral-N and rice residue additions: no additions, mineral-N only, low C-to-N residue, mid C-to-N, high C-to-N, low C-to-N + mineral-N, mid C-to-N + mineral-N, and high C-to-N + mineral-N. A second experiment consisting of a 90-day incubation of mineral-N and rice residue, amended to soil alone or in combination, to determine if input of mineral-N leads to more stable SOM formation when residues are amended to soil, if mineral-N is the predominant N-source in stable SOM when both are present, and whether mineral-N inputs may affect soil C sequestration. Mineral-N additions increased soil respiration regardless of residue quality, and microbial respiration increased with residue addition in the order Low C-to-N > Mid C-to-N > High C-to-N on a g^{-1} C added basis. The cumulative increase in C mineralization was significantly higher for the low C-to-N rice residue and mineral-N when normalized for C addition. Therefore C availability was important in modifying the effect of mineral-N on decomposition. The movement of C and N through the microbial biomass suggested that residue-C and residue-N were not linked through the decomposition pathway. Significantly more mineral-N was transformed into humin-N with residue input than when mineral-N was added alone. As microbial C and N are important precursors in the formation of stable SOM, the decoupling of the residue-C and N may suggest a direct role of mineral-N additions through fertilization or atmospheric deposition in the cycling and sequestration of C.

Objectives

1. To determine the contributions of residue-N and fertilizer-N in sequestered C in stable organic matter pools.
2. To quantify differences in C and N sequestration pathways between a conventionally managed and an organic agroecosystem.

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Approach and Research Procedures

The soil for this study was taken from a field cropped continuously with rice for at least 80 years in California. The soil is characterized by a complex of two soil series, Esquon (fine, mixed, superactive, thermic Xeric Epiaquerts) and Neerdobe (fine, mixed, superactive, thermic Xeric Duraquerts)

Plant Material

To facilitate $^{15}N^{13}C$-labeling of residues, rice plants (*Oryza sativa* L.) were grown in pots under controlled greenhouse conditions. Half of the pots were left unlabeled to produce non-^{15}N and ^{13}C-labeled rice residues grown under similar conditions. Nitrogen was applied with a separate solution of $(NH_4)_2SO_4$, labeled plants receiving 5.0 atom % ^{15}N-$(NH_4)_2SO_4$ and unlabeled plants receiving background 0.36 atom % ^{15}N-$(NH_4)_2SO_4$. The plants were labeled with ^{13}C-CO_2 in a climate-controlled chamber every two to three weeks for the first five months and once a week during the final two months before harvest. Samples were analyzed for their %C, %N, C:N ratio, atom %^{15}N and delta ^{13}C content determined

For a second experiment, rice straw residues with a range of C-to-N ratios were obtained from a field N fertilizer response trial. Low (45:1), mid (62:1), and high (99:1) C-to-N residue was chopped to 2 mm fragments before addition. Residue lignin % by weight was low (19%), mid (16%) and high (15%).

Soil Sampling

The top 10 cm of soil was sampled at 20 random positions throughout the field, air-dried to facilitate sieving to 4 mm; roots and large pieces of plant material were removed. Texture was 43% sand and 42% clay. To label the microbial biomass 30 g of soil were weighed into plastic specimen cups, followed by the addition of ^{15}N-$(NH_4)_2SO_4$ and ^{13}C D-glucose. A set of three treatments was amended with residues of low, mid and high C-to-N ratios. Each treatment received the same amount of residue-N, 5.0 kg residue-N ha^{-1} or equal to 112 kg residue ha^{-1} for the high C-to-N residue. A second set of three treatments were amended with the same amount of residue, but also received mineral-N at a rate of 5.0 kg N ha^{-1} to match the organic N treatments. There were two controls: soil without any additions and a control that received only mineral-N at a rate of 5.0 kg N ha^{-1}. Treatments were replicated five times.

On d 0, eight 30-g samples of soil with labeled microbial biomass were used to determine the initial values for ^{13}C and ^{15}N. The remaining samples were amended with their respective treatment additions. Residues were added to the specimen cups of $^{13}C^{15}N$-labeled soil, followed by either water or mineral-N solution to reestablish moisture content to 55% of water holding capacity. The soil samples were mixed, placed in a 970 mL Mason jar with 2 ml of water in the bottom to prevent desiccation, sealed with a jar lid containing a gas sampling septum. Mason jars were held in the dark at 25 ºC for 40 d. Two solutions of $(NH_4)_2SO_4$, with 5.0 atom % ^{15}N or with natural abundance 0.36 atom % ^{15}N, were the mineral-N sources for the incubation. Soil was amended with ^{15}N-labeled mineral-N only, $^{13}C^{15}N$-labeled residue only, unlabeled mineral-N and $^{13}C^{15}N$-labeled residue combined, or ^{15}N-labeled mineral-N and unlabeled residue together. There were four replicates and appropriate controls were included.

Treatments amended with residues received 0.2 ± 0.0001 g rice straw per 40 g soil and those amended with mineral-N received 3.4 mg N per 40 g soil. On day 0, five 40 g samples of soil were used to determine the initial, pre-incubation values for ^{13}C and ^{15}N, mineral N, microbial C and N, and also SOM fractions.

Analyses

On days 1, 2, 4, 8, 15, 30, 40 and 90, headspace CO_2 and $^{13}CO_2$ were determined. Soil microbial biomass was determined using the chloroform fumigation-incubation at 0 and 40 d. Soil was analyzed for NH_4^+ content and the ^{15}N content. Total CO_2 and NH_4^+ and their isotopic compositions were used to calculate the microbial biomass-C: $B_c = ((F_c - F_cK_1) - (UF_cK_2))/0$ where B_C is soil microbial biomass C, F_C is the CO_2 produced from the chloroform treated soils after 10 d of incubation and UF_C is the CO_2 produced in an untreated control sample over 10 d; values for K_1 and K_2 are 0.29 and 0.23, respectively. Microbial biomass-N was calculated as $B_N = (F_N - UF_N)/0.54$, where B_N is soil microbial biomass N, F_N is the NH_4^+ mineralized in the chloroform-treated soils and UF_N is the NH_4^+ mineralized in an untreated control sample.

The dilution (D) values were calculated to assess the amounts of dilution of the C and N isotope pools that occurred during the incubation. Values were calculated as follows:

$$D_N = (\text{atom\%}^{15}N_{final} - \text{atom\%}^{15}N_{background})/(\text{atom\%}^{15}N_{initial} - \text{atom\%}^{15}N_{background})$$

A similar equation was used for ^{13}C.

Residue-derived respiration was calculated using an isotope mixing equation:

$$Q_{res} = \frac{(Q_t \times (\delta_{soil} - \delta_t) + Q_{bl} \times (\delta_{soil} - \delta_{bl}))}{(\delta_{soil} - \delta_{res})}$$

where Q_{res} is the residue-derived CO_2-C respired, Q_t is the total measured respiration of the treatment, Q_{bl} is the measured blank, δ_t is the delta PDB of the measured total respiration, δ_{res} is the δ-PDB of the added labeled residue and δ_{soil} is the δ-PDB of CO_2 derived from the soil in the soil-only control treatments, calculated from the equation:

$$\delta_{soil} = \frac{(Q_t \times \delta_t - Q_{bl} \times \delta_{bl})}{Q_t}$$

Soil-derived respiration from treatments with added labeled residue is calculated as total respiration minus residue-derived respiration.

Soil Organic Matter Fractionation

After 90 days, soil (~15 g) from each treatment replicate was air-dried prior to SOM fractionation. A physical separation was undertaken to separate undecomposed organic matter (>500 μm, the size of the smallest residue pieces added at the beginning of the incubation), particulate organic matter (POM; 53-500μm), and SOM <53 μm. A chemical fractionation of the <53 μm physical fraction consisted of alkali extraction of fulvic and humic acids from the humin, followed by acid precipitation of the humic acid from the fulvic acid.

The fraction (f) of C derived from residue in the microbial biomass or any SOM fraction was calculated by:

$$f = \frac{\delta_{end} - \delta_{initial}}{\delta_{input} - \delta_{initial}}$$

where δ_{end} is δ-PDB of the treatment C, $\delta_{initial}$ is the δ-PDB of the initial MBC or fraction, and δ_{input} is the δ-PDB of the labeled residue. The amount of residue-derived C in the microbial biomass or SOM fraction is the product of f × [MBC or total fraction C]. The residue or mineral derived N was first calculated as N derived from labeled source ($\%N_{dfl}$):

$$\%N_{dfl} = \frac{\text{atom\%excess}_{end}}{\text{atom\%excess}_{input}} \times 100$$

where $\text{atom\%excess}_{end}$ is the atom % ^{15}N measured for the treatment minus a background of the initial atom % for that fraction, and $\text{atom\%excess}_{input}$ is the atom % ^{15}N of the residue or mineral-N input minus a background of the initial atom % for that fraction. The amount of residue or mineral-derived N is the product of the $\%N_{dfl}$ × [MBN or total fraction N]. Percent recoveries in MB or fractions were determined by dividing residue derived C by added C or residue/mineral derived N by added N, and multiplying by 100%.

The net C and N that moved into or out of designated fractions at the end of 90 days that is not derived from inputs was calculated as C or N transfer:

$$\text{Transfer} = F_{end} - F_{ini} - F_{dfi}$$

where F_{end} is the amount of C or N in the fraction at the end of 90 days, F_{ini} is the amount of C or N in each fraction from a set of initial, preincubation soil samples, and F_{dfi} is the amount of C or N in each fraction derived from inputs.

Statistical Testing

All statistical analyses were performed using the SAS system version 8 (SAS Institute Inc., NC). Data was analyzed using ANOVA (the GLM procedure) with residue, mineral-N input, or combinations as treatments. Significant differences among treatments and controls were determined using Tukey's Studentized Range Test ($P < 0.05$, unless otherwise noted).

Results

Respiration was higher throughout the experiment when residue was added (*fig. 1*). Mineral-N addition alone did not significantly change respiration. Overall, treatments receiving residue with a high C-to-N ratio respired the most, followed by residue with the mid C-to-N and residue with the low C-to-N ratio. Treatments with residue plus mineral-N additions followed the same pattern. When the respiration data is expressed in g CO_2-C g^{-1} of C added (*fig. 2*), the treatments receiving mineral-N in addition to residue still showed significantly higher respiration, but the effect of mineral-N on C mineralization for the different residues is altered, with more g CO_2-C respired g^{-1} of C added for the low C-to-N residue. Increase in respiration with mineral-N addition was significantly greater (g CO_2-C g^{-1} of C added) for low C-to-N residue than high C-to-N residue, with mid C-to-N falling in between (*table 1*).

Control treatments showed substantially less mineralization of original microbial ^{13}C than the other treatments (*fig. 3*). The other treatments were not significantly different, except for the high

C-to-N ratio residue without mineral-N. For this treatment, significantly more ^{13}C was mineralized compared to the other treatments. This turnover of ^{13}C contributed to the total C respired (*fig. 2*), but microbial-^{13}C respiration was small compared to total respiration.

The microbial biomass C pool (MBC) either remained the same size or was larger in the treatments and controls at 40 d (*fig. 4a*). Both control treatments and the low C-to-N residue treatment were the same size of MBC as the initial pool. In all other treatments, MBC was significantly higher at 40 d. The addition of mineral-N to the mid and high C-to-N treatments did not show significantly more MBC at day 40.

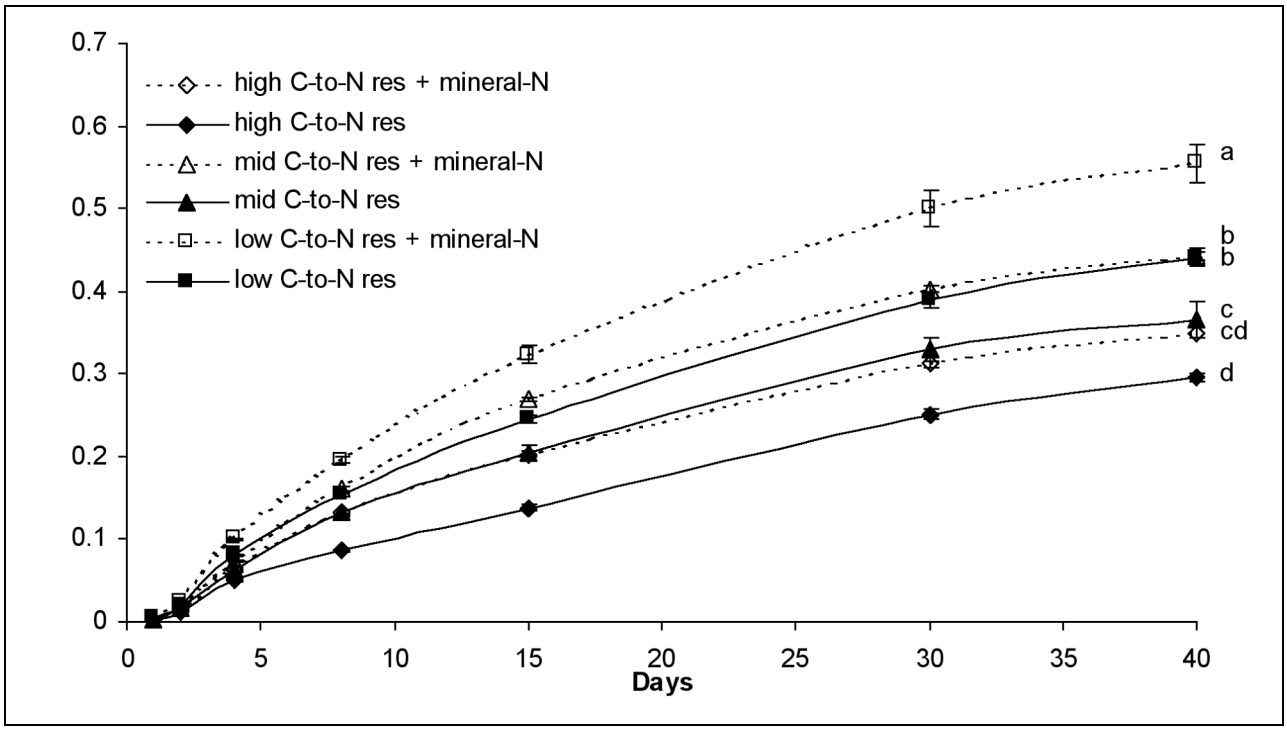

Figure 1. Cumulative CO_2 respiration during a 40-day incubation of different qualities of rice residue with or without mineral-N. Bars show standard errors.

For both controls and treatments, except for the low C-to-N residue without mineral-N, retention of MB^{13}C was not significantly different and averaged 73% of the initial MBC after 40 d (*fig. 5a*). The low C-to-N residue without mineral-N treatment showed a ^{13}C recovery of only 11%. However, this treatment did not show a significant difference in ^{13}C mineralization from all other treatments (*fig. 3*).

The effective dilution of MBC pools is an indicator for the turnover of C through the microbial biomass. The resulting D_C values (Fig. 6a) show the least dilution of the MBC pool occurring in the controls when no residue or mineral-N was added. Small differences emerge among the treatments, mostly insignificant, with a slightly higher dilution in high C-to-N residue treatments, which became significant when mineral-N was added.

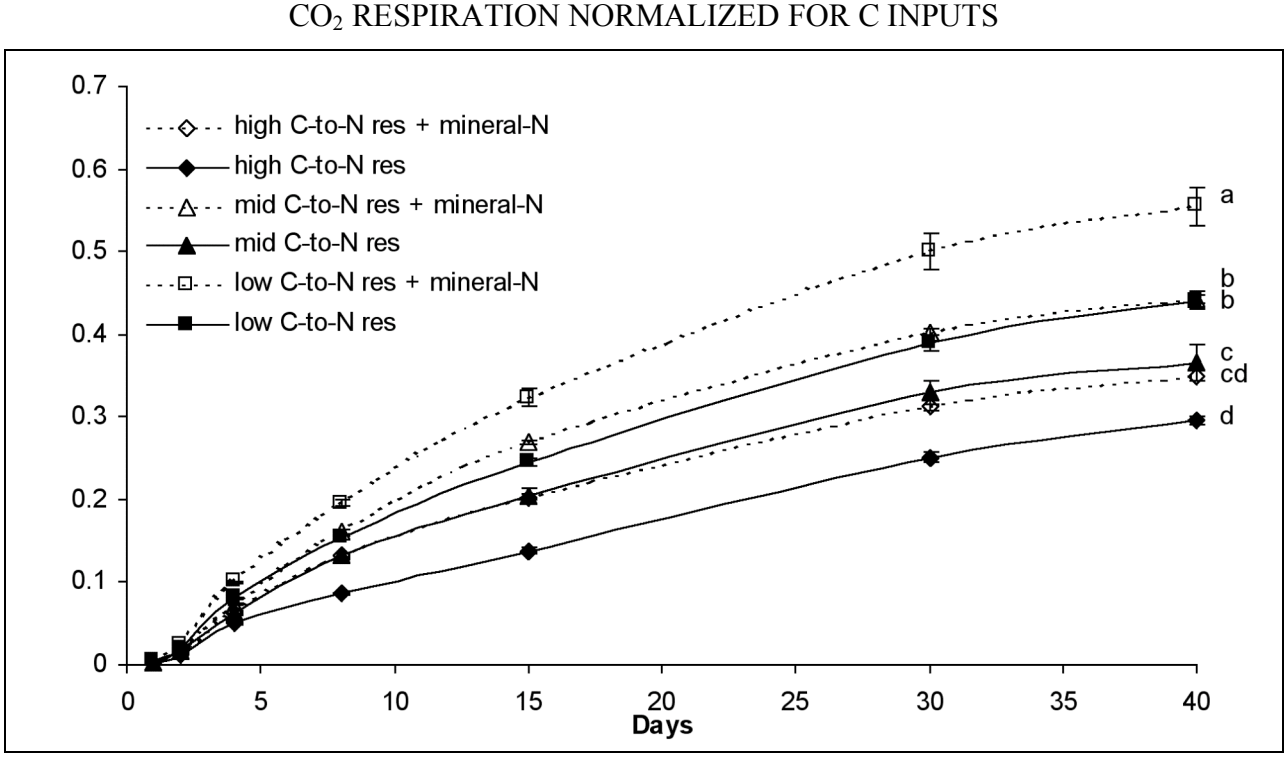

Figure 2. Cumulative CO_2 respiration during a 40-day incubation of different qualities of rice residue with or without mineral-N, normalized for C added. Bars show standard errors.

Table 1. *Means and statistical significances of C mineralization increases with mineral-N addition for a range of rice residue C to N ratios, totals after a 40-day incubation.*

Residue	Increase, cumulative (μg CO_2-C respired g^{-1} soil)	Increase, normalized for C-input (μg CO_2-C respired μg^{-1} C input)
Low C:N	258 (a)	0.114 (a)
Mid C:N	239 (a)	0.077 (ab)
High C:N	266 (a)	0.054 (b)

[a]n=5, letters in parentheses beside means denote statistical differences indicated by Tukey's Studentized Range Test ($P > 0.05$).

After 40 days, adding residue alone either maintained or decreased the initial MBN pool size. However, the addition of mineral-N with residue showed a larger MBN pool for all C-to-N treatments at 40 days. The greatest effective increase in the MBN pool was observed when mineral-N and residue were added together, but the C-to-N ratio of the residue did not lead to significant differences (*fig. 4b*).

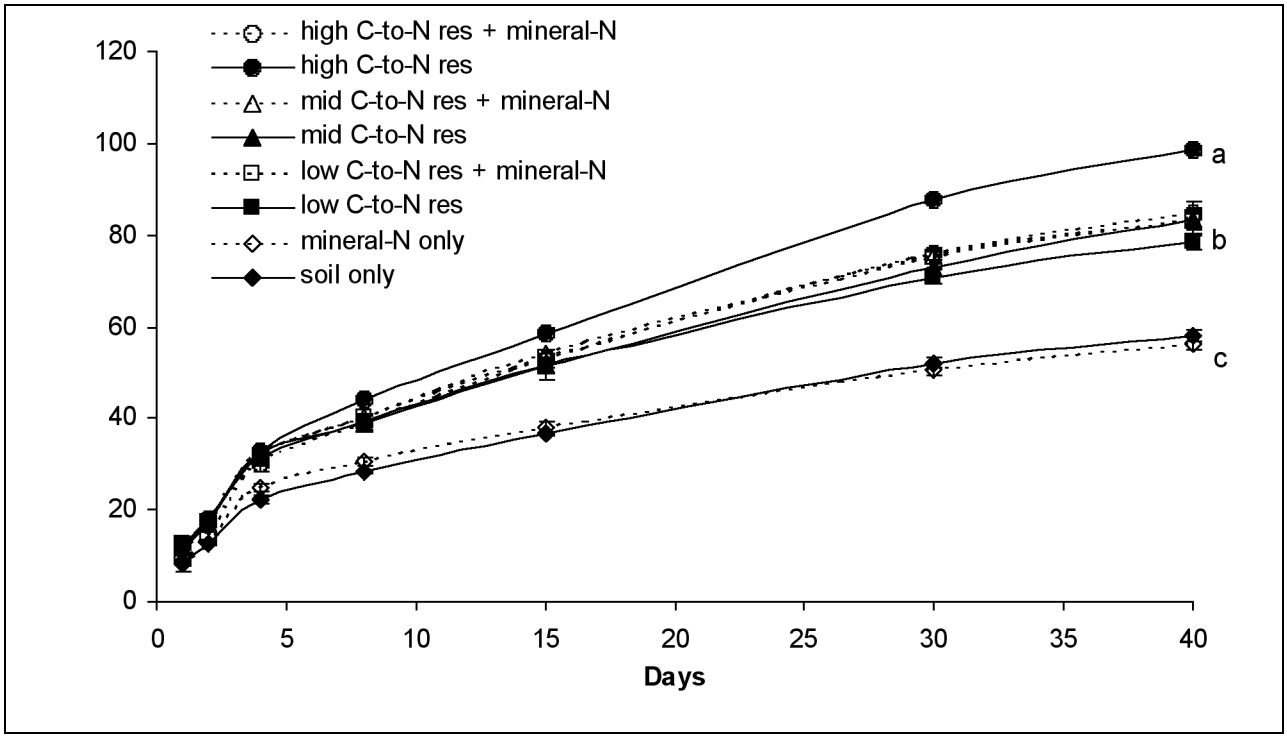

Figure 3. Cumulative CO_2 respiration of microbial derived C during a 40-day incubation of different qualities of rice residue with or without mineral-N. Bars show standard errors.

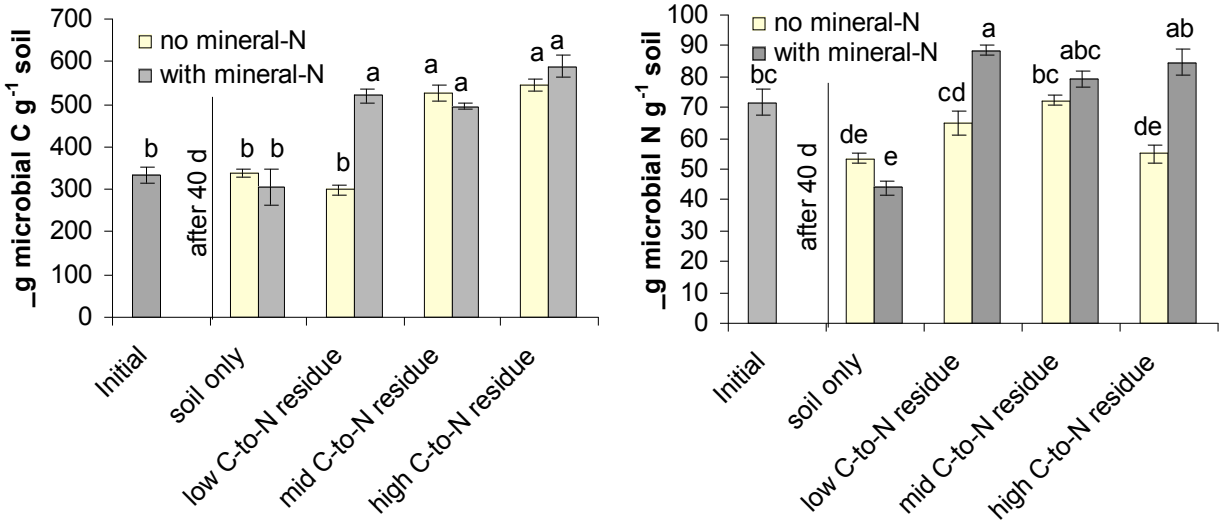

Figure 4. Microbial biomass initially and after a 40-day incubation of different qualities of rice residue with or without mineral-N.

Since the microbial biomass present at time 0 was ^{15}N labeled, after 40 d the % of ^{15}N remaining in the microbial biomass (*fig. 5b*) gives an estimate of how much original microbial biomass N was conserved. The average recoveries of original ^{15}N in the microbial biomass were

high, over 47% for all treatments (*fig. 5b*). The highest recoveries were shown among treatments with residue plus mineral-N added. The MBN of these combination treatments were significantly higher than of their residue-alone counterparts, except for the mid C-to-N residue. Mid C-to-N residue without mineral-N showed similar conservation of original MBN as the other treatments receiving mineral-N. The lowest recovery of ^{15}N was for the mineral-N alone treatment.

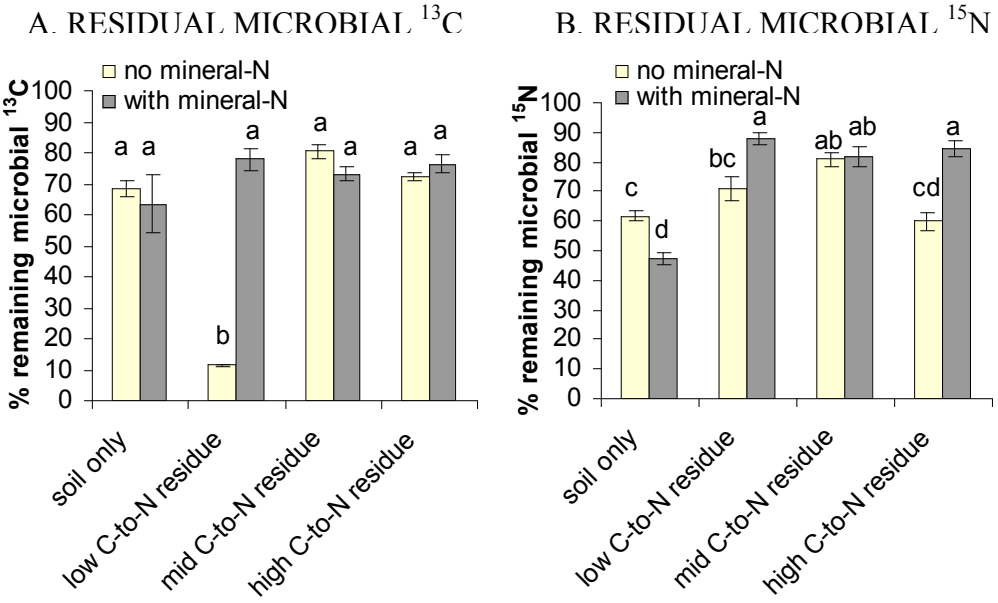

Figure 5. Retention of microbial ^{13}C and ^{15}N, calculated as percent remaining of the initial isotope concentration after a 40 d incubation of different qualities of rice residue with or without mineral-N.

Patterns and magnitudes in $MB^{15}N$ pool dilution were similar across C-to-N ratios (*fig. 6b*). Adding residue, mineral-N, or both combined caused a dilution in the $MB^{15}N$. The mineral-N control diluted the pool to the same degree as for each of the treatments that only received residue. For treatments where a combination of residue and mineral-N were added, the difference in the ^{15}N dilution from the control soil was similar to the sum of the ^{15}N dilution effects if residue alone or mineral-N alone were added.

To evaluate differences in the total, residue-derived and soil-derived CO_2-C respired in the absence or presence of mineral-N, the treatments that received $^{13}C^{15}N$-labeled residue alone or with mineral-N were compared (*fig. 7*). Total respiration was initially higher with the input of mineral-N in combination with residue. The difference with mineral-N input was significant through day 60, but ceased to be significant at the end of the incubation on day 90. Soil-derived respired C was consistently similar with residue alone or residue and mineral-N together for the entire incubation. Therefore, no significant priming of the soil C occurred due to added N. This is further supported by the isotope data (*fig. 3-1*) that shows no significant differences at any sampling date in soil-derived CO_2-C respiration between the mineral-N + residue and the residue-alone treatments.

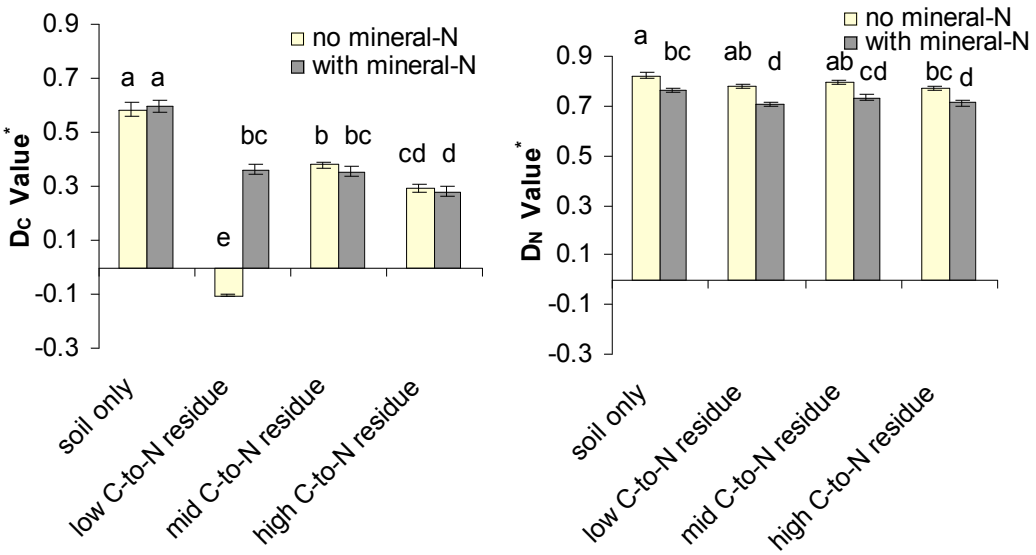

Figure 6. Isotopic dilution of microbial biomass assessed after a 40-day incubation of different qualities of rice residue with or without mineral-N.

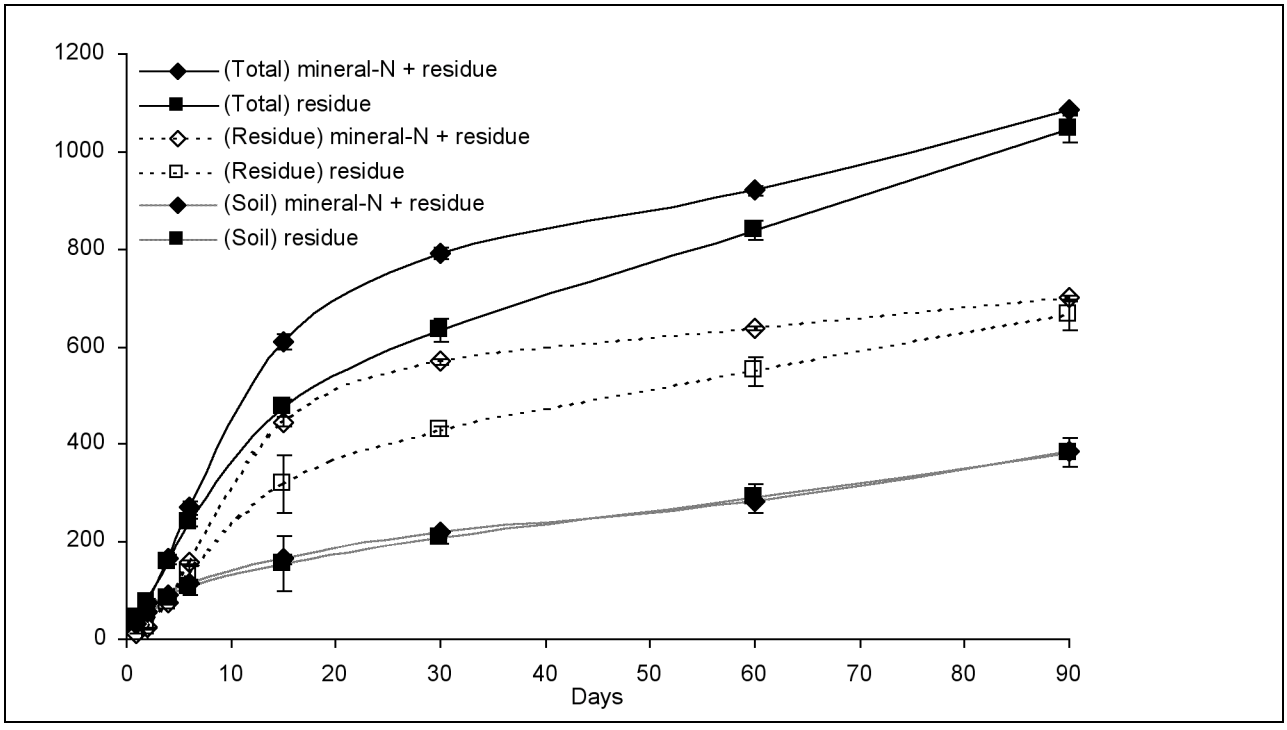

Figure 7. Cumulative total, residue-derived and soil-derived respiration for a 90-day incubation of treatments with ^{13}C-labeled residue with and without mineral-N input.

Great variability was seen in the microbial biomass measurements (*table 2*). None of the comparisons between treatments were significantly different at $P<0.05$. A larger percentage of residue-derived C transformed into MBC on day 6 than day 90. There was a trend of more residue-derived C in the mineral-N + residue treatment than the residue-alone treatment on both day 6 and day 90 although neither was statistically significant. Microbial biomass nitrogen was also very variable and conclusions drawn from the obtained data should be very cautious.

Total recovery of added C was 84.2 ± 3.2% for the mineral-N plus residue treatment and 90.8 ± 2.7% for the residue alone treatment (including all SOM fractions and respired C). Most of the added C was respired, 39.2 ± 0.4% for the mineral-N plus residue treatment and 37.2 ± 1.0% for the residue alone treatment, but most of the C remained in the POM and SOM fractions (24.6 ± 1.2% for mineral-N and residue, and 25.1 ± 1.5% for residue alone). The remaining C was undecomposed, in the >500 µm fraction. When comparing the % recovery of added residue-C across the five SOM fractions (*fig. 8*), a trend of less residue-C recovered in all the fractions was observed with mineral N input, except in the humin, where a small but significantly higher amount of residue-C is recovered.

Table 2. *Microbial biomass C at day 6 and day 90, and microbial biomass N at day 90: totals, totals derived from source, and transferred.*[†]

Fraction	Microbial Biomass C		Microbial Biomass N
Treatment	Day 6	Day 90	Day 90
	µg g^{-1} soil		µg g^{-1} soil
Total			
Mineral-N	196 ± 32 (5)	165 ± 40 (4)	53 ± 35 (3)
Mineral-N + Residue	273 ± 20 (10)	242 ± 32 (7)	22 ± 11 (7)
Residue	262 ± 24 (10)	234 ± 36 (8)	29 ± 5 (8)
Residue-Derived			
Mineral-N	NA	NA	NA
Mineral-N + Residue	122 ± 14 (5)	52 ± 7 (3)	2.1 ± 0.4 (3)
Residue	110 ± 11 (5)	47 ± 8 (4)	2.4 ± 0.4 (4)
Mineral-N Derived			
Mineral-N	NA	NA	29 ± 27 (2)
Mineral-N + Residue	NA	NA	12 ± 9 (3)
Residue	NA	NA	NA
Transferred			
Mineral-N	121 ± 32 (5)	89 ± 40 (4)	29 ± 24 (2)
Mineral-N + Residue	91 ± 26 (5)	126 ± 51 (3)	-5.1 ± 9 (6)
Residue	85 ± 24 (5)	95 ± 49 (4)	8.2 ± 6 (4)

†Standard errors shown for each data set, number of replicates shown in parentheses. NA, not applicable.

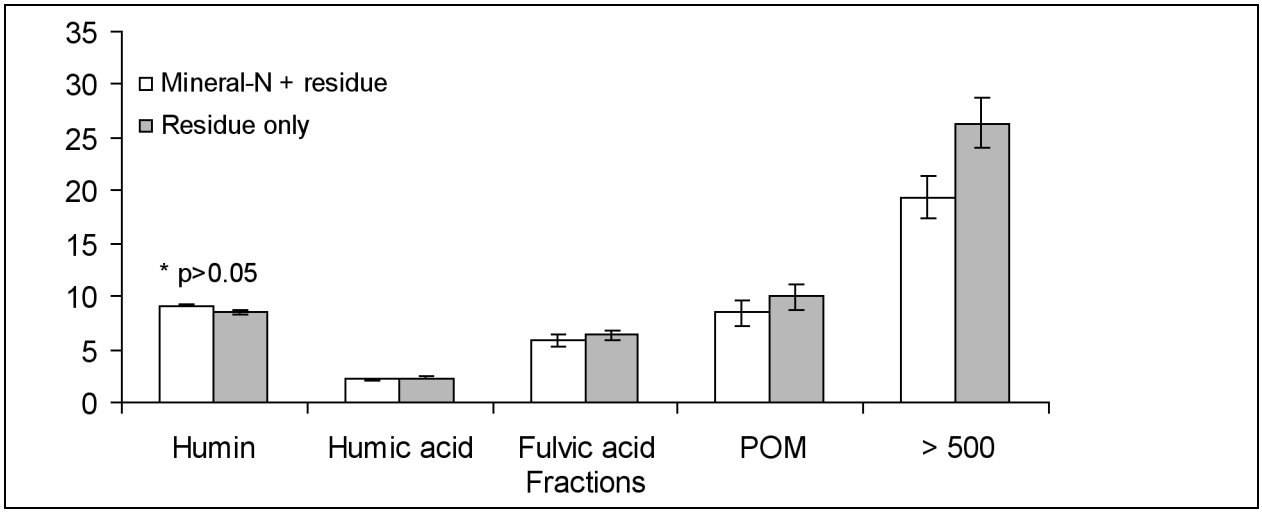

Figure 8. Percent recovery of added residue-C in SOM fractions after a 90-day incubation of $^{13}C^{15}N$-labeled residue with and without mineral-N input.

Total C was not significantly different among treatments for any of the SOM fractions. Much (14.7 - 33.2%) of the original added residue-C after 90 days remained in the >500 μm, undecomposed fraction *(fig. 9*). In the humin fraction (*fig. 9*), total C per g soil was not significantly different between the mineral-N + residue and residue alone treatments, but mg of residue-derived C per g soil was greater in the mineral-N + residue treatment. Changes in the SOM fraction sizes at the end of the experiment as compared to initial values that cannot be attributed to labeled additions suggested that during the course of the incubation some C moved between the different soil fractions. A loss of original soil C in the >500 and humic acid fractions was observed, with a gain in all other fractions (except for the POM fraction from the mineral-N and residue treatment).

Overall, more of the added residue-N than mineral-N was recovered in all the treatments for all the SOM fractions (*fig. 10*). There was also a trend of more mineral-N recovery in all fractions with residue addition, with significantly more recovered in the humin and POM fractions. Mineral-N, added alone, was incorporated the least into SOM fractions; it remained mostly in the inorganic N fraction (*fig. 10*). Adding mineral-N with residue did not lead to significant change in residue-N mineralization evidenced in the inorganic-N pool. Overall, more residue-N was recovered in the SOM fractions when it was not amended with mineral-N.

Total N content was not significantly different between treatments for any of the fractions, except for the >500 fraction undecomposed fraction (*fig. 11*). Much of the original added residue-N and mineral-N after 90 days remained in the >500 fraction. In addition, some of the added mineral-N transferred to the POM fraction, with significantly more transferred into POM in the mineral-N plus residue treatment compared to the mineral-N alone treatment.

Changes in the SOM fraction sizes at the end of the experiment as compared to initial values that cannot be attributed to labeled additions suggested that during the course of the incubation some N moved between the different soil fractions. Similar to the C transfer (*fig. 9*), a net loss of original soil N also occurred in the >500 fraction (*fig.11*), but the humic acid fraction overall showed a small gain, except for the mineral-N treatment. Loss of original N from the inorganic-

N fraction in the residue alone treatment was shown, as well as a gain of original N in the inorganic-N fraction for the mineral-N and residue treatment. Analysis of the humin fraction Ng[1] soil from the mineral-N vs. residue-N sources (*fig.11*) revealed that a significantly greater amount of mineral-N becomes part of the humin-N when mineral-N and residue are added together. Also, significantly less residue-N contributed to the humin in this treatment, as compared to when residue was added alone. Total humin-N content, was not significantly different among the treatments.

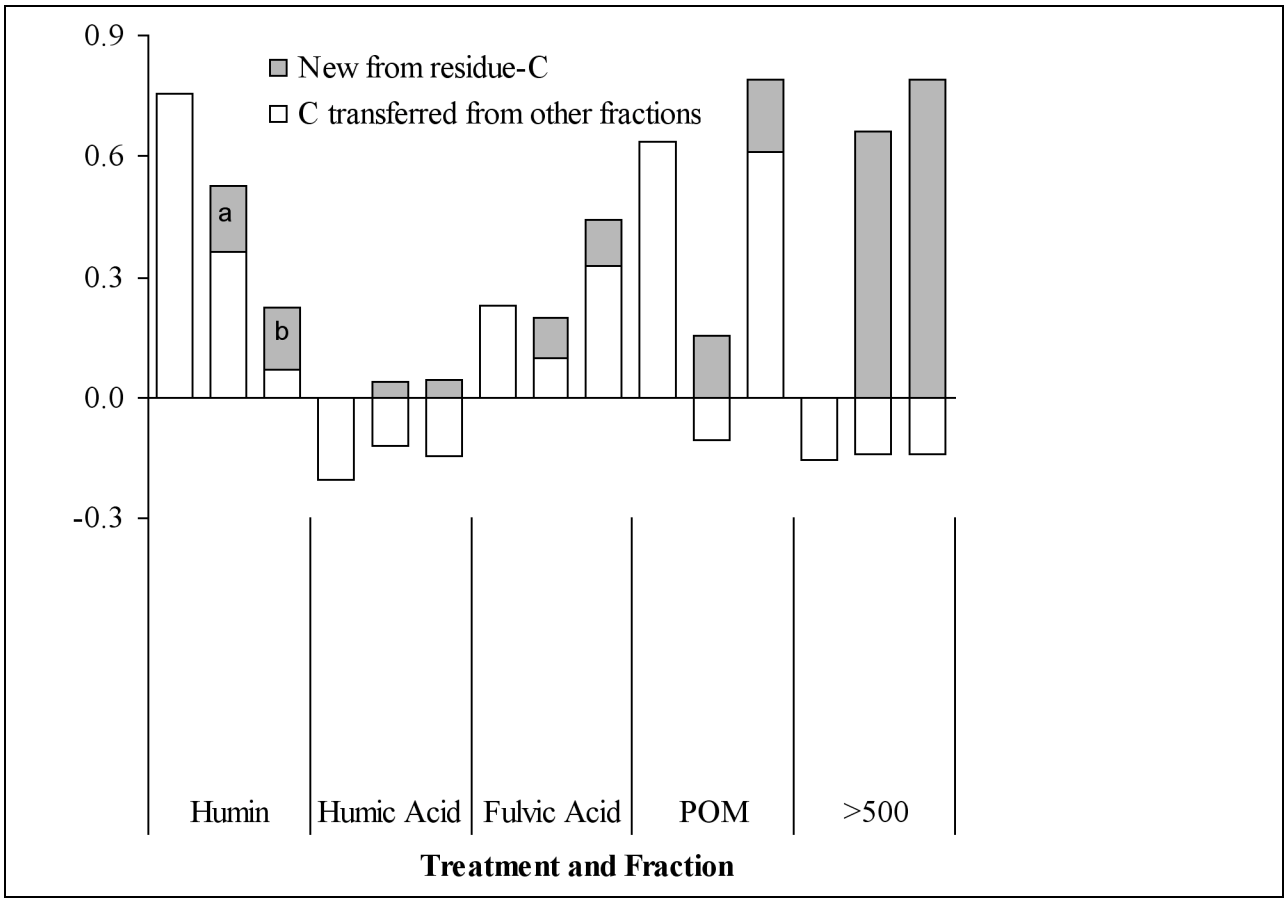

Figure 9. Milligrams C per g of soil in SOM fractions after a 90-day incubation derived from residue or original soil C that moved between fractions.

The relative contributions of residue-N and mineral-N to the humin fraction after 90 days (*fig. 12*) indicated that more residue-N was incorporated into the humin as compared to mineral-N, once the values are corrected for the amount of N added. There was no significant difference in the amount of residue-N incorporated into humin when mineral-N was present. But, more mineral-N was transformed into humin in the presence of residue.

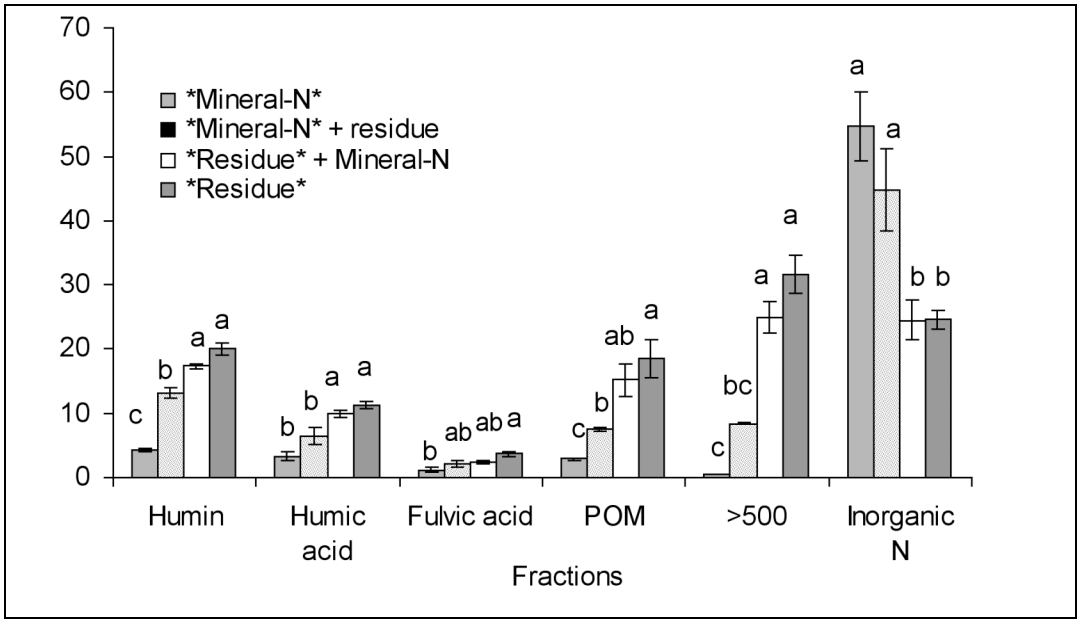

Figure 10. Percent recovery of residue-N or mineral-N in SOM fractions after a 90-day incubation with or without mineral-N inputs, ^{15}N-labeled mineral-N, and unlabeled residue with labeled mineral-N inputs. ^{15}N-labeled sources indicated in legend with asterisks.

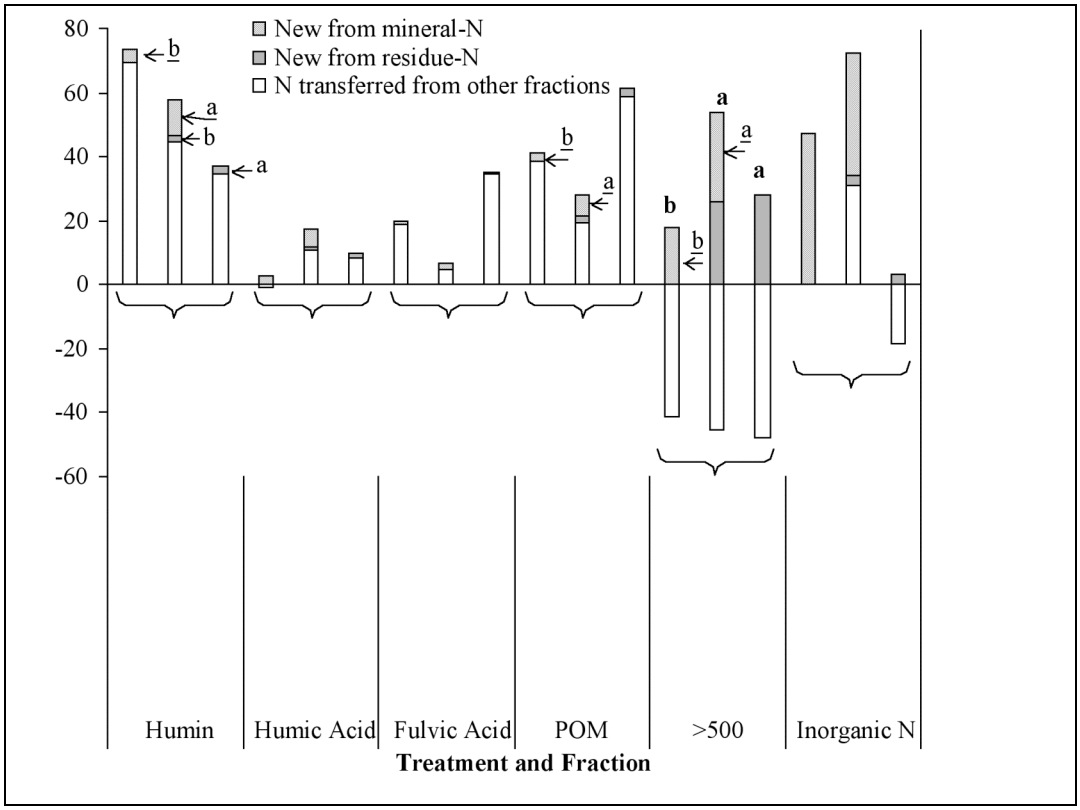

Figure 11. Mg N per g of soil in SOM fractions, after a 90-day incubation, derived from mineral-N, residue or original soil N that moved between fractions.

Discussion

The effect of residue quality and mineral-N on microbial respiration and transformation

Cumulative respiration (*fig. 1*) was the highest when more residue-C was added in the high C-to-N residue treatments as a result of more C substrate available for microbes. Our 40-day experiment shows a positive effect of mineral-N on short-term residue decomposition. Possibly this increased activity may have been a pH effect, caused by the strong increase in pH caused by urease hydrolyzing the urea (Berg and Matzner 1997), although this pH effect is only temporary, but others have shown that mineral-N can accelerate the initial decomposition of residues, and in the more advanced stages of decomposition the opposite can occur (Leuken et al.1962; Fogg 1988; Berg and Matzner 1997). Chronically applied mineral-N increases microbial cellulase activity, but when mineral-N is added to high-lignin litter the activity of microbial lignin-degrading enzymes declines (Carreiro et al. 2000). Thus, once the residue cellulose is exhausted, lignin can control decomposition in the later stages, and a suppression of C-mineralization results. This phenomenon was observed within 40 days of a long-term experiment (Leuken et al. 1962). However this phenomenon was not apparent during our 40-day incubation, thus the effect of mineral-N on lignin does not appear to be important in our short-term experiment. Even though the low C:N residue had the greatest lignin content, with mineral-N input it showed a similar magnitude increase in total respiration to the other residues (*table 1*). In forest systems, tree litter has a higher lignin content compared to rice straw, i.e., 20-30%. Mineral-N additions may increase leaf litter decomposition more in ecosystems characterized by low-lignin litter than in those systems characterized by high-lignin litter (Hobbie 2000). Therefore in most fertilized agroecosystems mineral-N may have a greater effect on residue decomposition than in forest systems, since lignin may not play the same rate-determining role.

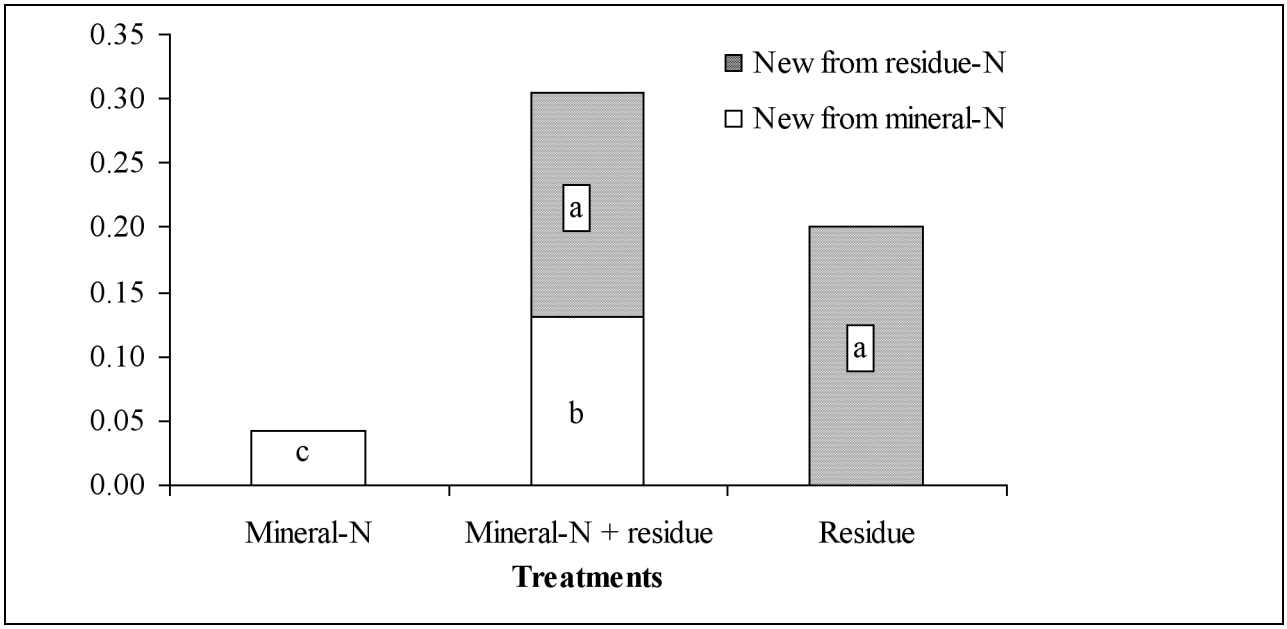

Figure 12. New N in stable humin fraction derived from added residue or mineral-N. Significant differences in N derived from residue and fertilizer are denoted by letters.

Added mineral-N by itself did not cause more soil-C mineralization, and therefore there was no priming of the soil-C. The increase in respiration with mineral-N and residue combined may be due to a priming of the residue itself. Leuken et al. (1962) found that in contrast to all other residues tested, N additions to cellulose caused a continuous and large increase in residue decomposition. In addition, Azam et al. (1985) found indirect evidence of increased mineralization of residue-N in the presence of added mineral-N. Mineral-N, therefore, may increase mineralization of both residue C and residue N.

After normalizing for C inputs (*fig. 2*), a reversal in the order of residues with the least C-mineralization in the high C-to-N treatment g^{-1} C input clearly showed that carbon availability controlled decomposition in this study. Since the residue additions were normalized for N addition, and as the data presented in figure 2 is normalized for C addition, differences in residue treatments can be attributed to inherent differences in the quality of residues. We expected that high C-to-N residue would be more N-limited and therefore respond the most to added mineral-N, but the low C-to-N treatment showed the greatest increase in C mineralization with mineral-N addition (*table 1*). This is especially unexpected since this residue had slightly more lignin than the others. But, since less C was added with the low C-to-N residue, therefore the effect of mineral N g^{-1} C input was greatest for this residue. Horwath and Elliott (1996) found that when they incubated ryegrass straw with $(NH_4)_2SO_4$ in soil for 45 days, the more inorganic N that was added, the more cumulative C mineralization (mg CO_2-C g^{-1} straw) was reduced. In our study, the addition of mineral-N with residues caused an increase in respiration, the greatest increase shown for the low C:N ratio residue (*fig. 2*). This finding is counterintuitive when considering that by adding mineral-N in combination with residue the C-to-N ratio of the inputs was reduced by half.

The fact that high C-to-N residue treatments respire less C $unit^{-1}$ C added is probably due to the residue's higher chemical complexity, which would make it more difficult for microbes to decompose. However, with simultaneous addition of mineral-N and residue, more C is mineralized per residue-C added. But, in contrast to our hypothesis, the addition of an accessible pool of mineral-N increased C-respiration g^{-1} C added the most for the highest quality rice residue used in our study.

Since we ^{13}C-labeled the original microbial biomass, cumulative ^{13}C-CO_2 respiration over time (*fig. 3*) was an indicator for the turnover of what was microbial biomass at time 0. The absence of significant differences in respiration of microbial-derived C when mineral-N was added excluded the possibility that differences in the turnover of the original microbial biomass might affect total respiration. Only soil treated with high C-to-N ratio residue by itself showed any significant difference from the others, and this increase was small when compared to the overall cumulative respiration in this treatment (*fig. 1*).

At the end of 40 days any differences in the size of the MBC brought about by mineral-N additions, if they had occurred, disappeared for mid and high C-to-N residues (*fig. 4b*); however, the cumulative respiration data showed that earlier mineral-N did stimulate the microbial biomass to grow and be more active (*fig. 1*). A difference in the size of the MBC for the low C-to-N residue with mineral-N addition was evident at day 40 (*fig. 4b*), with the residue-only treatment MBC similar in size to the initial MBC. This treatment received the least residue-C, and the microbial biomass may have mineralized all available C by the end of the incubation, causing the microbial biomass to starve and decrease in size. The size of the microbial biomass

for the low C-to-N residue must have increased after residue addition and then decreased back to its initial size since more C is mineralized in this treatment than for the controls (*fig. 1*). Control MBC was consistent with the initial samples at 40 days (*fig. 4b*). But it was noteworthy that with mineral-N added, MBC remained high for the low C-to-N residue (*fig. 4b*), and during the 40 days more C was mineralized (*fig. 1*), suggesting that with added mineral-N the microbial biomass was able to access more of the C in the low C-to-N residue and therefore its size lasted longer and more residue-C was mineralized. Jacinthe et al. (2002) found that applying fertilizer (N-P-K) with wheat residue led to a 1.5-fold increase in C mineralized, and a 1.3- to 4-times increase in the metabolic quotient defined as unit of C mineralized per unit of MBC d^{-1}. They proposed that these increases are due to an alteration in the microbial community composition whereby fertilization leads to a lower efficiency of C processing. The implications of the effects of mineral-N on C sequestration in ecosystems receiving mineral-N are difficult to gauge because it ultimately depends on whether this increased C was mineralized at the expense of forming stable SOM. The lowest quality residue used in this study without mineral-N stimulated respiration of the original MBC (*fig. 3*). It is possible that the microbial community composition changed in the presence of this complex substrate.

Unlinking residue-C and residue-N through the microbial biomass

Many have reported greater conservation of N in the soil rather than C, since there is greater internal cycling of N but C is continuously lost through microbial respiration (e.g., Angers et al. 1997; Ladd et al. 1981). Our data shows that, when a C-source was present with mineral-N input, more initial MBN was conserved than without mineral-N (*fig. 5b*). Figure 5a shows a consistent level of initial MBC conservation for all treatments after 40 days, regardless of mineral-N addition, except for the low C:N residue alone treatment. Low ^{13}C retention (*fig. 5a*) and a negative D_C value (*fig. 6a*) in the low C-to-N residue alone treatment suggest that more ^{13}C was cycled into the bulk soil than the others, or that there was some other isotope-depleting mechanism. However, a satisfactory explanation for this data was not forthcoming, thus this treatment should be interpreted with caution.

For mid and high C-to-N residues, the addition of mineral-N did not result in increased MBC (*fig 4a*). However, treatments that received both mineral-N and residue showed a trend of increased MBN (*fig 4b*), with a significant increase for low and high C-to-N residues. This increase in N might be indicative of luxury N uptake. Fog (1988) suggested when mineral-N is added during straw decomposition, the luxury uptake of N by microbes should be considered.

Residue-C and residue-N were not proportionally linked when transformed by the microbial biomass. Initial microbial C is more highly diluted after 40 days of incubation than microbial N (*figs. 6a and 6b*). Since residue-C and residue-N do not necessarily travel through the decomposition pathway together, and mineral-N is a more readily available N-source, mineral-N could be preferentially taken up into the microbial biomass and transformed into stable precursors of SOM over residue-N. It has been shown that a significant fraction of recently immobilized N derived from fertilizer or atmospheric deposition is transformed into insoluble components of microbial tissues (e.g., fungal melanins; He et al. 1998). The formation of these insoluble components in the microbial biomass remains important precursors leading to the formation of stable SOM and its concurrent sequestration of C. The microbial biomass N pool is not only an important pool through which N is associated with stable SOM, it is also a labile pool that may reduce losses of N during periods of low plant demand and could quickly become

available to plants (Bremer and van Kessel 1992). With mineral-N input, we showed more total MBN (*fig. 4b*) and more initial MBN conservation (*fig. 5b*). Further study is required to determine the effect of mineral-N on N sequestration both for SOM formation and long-term soil fertility, but our results indicate the potential of a decoupling of residue-C and residue-N through the microbial biomass.

Microbes will take up whatever N and C is most readily available to them, even if the two nutrients come from different sources. Smith et al. (1989) took a different approach than that we used here. They found that adding glucose with ^{15}N and ^{14}C labeled organic material caused nearly complete assimilation of ^{15}N into microbial biomass but had no effect on assimilation of ^{14}C. Organic N could be assimilated into the microbial biomass without organic C from the same source.

Although the residue C-to-N ratio has been used as a reliable indicator of decomposition potential and residue-N mineralization (e.g., Nicolardot et al. 2001), fertilizer and atmospheric mineral-N inputs add another layer of complexity to SOM formation that requires further study to fully understand. In models of decomposition and SOM formation, the impact of mineral-N inputs should be taken into account. While residue-C is the source of C leading to the formation of stable SOM, when mineral-N is added, not only is residue decomposition and microbial transformations modified, residue-N is no longer the sole source of N for microorganisms to assimilate into stable precursors of SOM.

Mineral-N increases total evolved CO_2 by increasing residue-C mineralization without raising soil-C mineralization (*fig. 7*). This is only an initial response, since the residue treatment attains the same level of total respired C as the mineral-N + residue treatment by the end of the 90-day incubation. This finding is in concordance with residue and inorganic-N addition experiments performed by Lueken et al. (1962), who found that lowering the C:N ratio of organic matter inputs with mineral-N to 10:1 increased the CO_2 production for the first few weeks compared to those without mineral-N input (in the current experiment the C:N ratio of inputs is only lowered to 18:1). However, between 30 and 150 days CO_2 production was lowered for mineral-N amended soils, and the result was similar total evolved CO_2 for both treatments. Thus, total respired C alone does not indicate whether mineral-N inputs cause more or less residue-C to be converted into SOM, since at the end of 90 days there is no difference in the amount of C lost by mineralization.

Jenkinson and Rayner (1985) suggested that added mineral-N may be filling an initial need by microbes to help decompose the straw material with a wide C:N ratio. There is also evidence that in the early decomposition of plant material, solubles and nonlignified holocellulose are decomposed, and mineral-N inputs stimulate this process (Berg and Matzner 1997). This is an advantage only initially; after 30 to 60 days (*fig. 7*) the difference in residue-derived CO_2 mineralization begins to narrow. At this time, added mineral-N may have been immobilized and the competitive advantage given the microorganisms in this treatment is diminished. But in order to respire an equivalent amount of C, the slower-growing microorganisms (such as fungi) that are able to thrive on residue with high C:N ratio when it is all that is available must overtake the respiration flush that occurred in the beginning of the incubation with the mineral-N input. Slower-growing microorganisms that will decompose high C:N residue when it is the only substrate available will take longer to establish themselves, and also their enzyme production is inhibited by mineral-N inputs. Many studies have shown that mineral-N inhibits the later-stage

decomposers of lignin (Henriksen and Breland 1999; Carreiro et al. 2000; Saiya-Cork et al. 2002).

Microbial C and N

The long-term depression in microbial respiration due to N inputs (Söderström et al. 1983) has long been attributed to decreases in microbial activity. Ågren et al. (2001) elaborated on this further and proposed that N-inputs cause (1) an increase in decomposer efficiency, (2) a more rapid formation of recalcitrant material and (3) a decreased growth rate of the decomposers. These observations are not universal, since an increase in the metabolic quotient with fertilization has also been shown (Jacinthe et al. 2002), suggesting that microbial metabolism was more inefficient with mineral-N inputs. In the present experiment the MBC for the mineral-N plus residue treatment at day 6 and day 90 is greatest compared to the other treatments (*table 2*). However, since there is no significant difference, I cannot draw conclusions about how mineral-N may have affected microbial growth.

More residue-derived C is in the MBC for the mineral-N + residue treatment vs. residue alone on both days, however, since this result is not statistically significant, it is difficult to draw firm conclusions from the data. At day 90, a drop in the residue derived-C in the MBC has occurred showing microbial turnover of new C during the incubation. New residue-C was incorporated into the MBC, thus microbial products were formed and transformed that contain new residue-C, and recalcitrant products contributed to changes in the hummified SOM content.

Residues in microbial biomass measurements tend to produce much variability in results. It was my hope that using the chloroform-fumigation extraction method with a control would sufficiently mitigate this variability (Horwath and Paul 1994); however, this was not the case. Pieces of residue caused a large amount of sampling error between control and fumigated samples in the quantitative microbial determinations, compared to the relatively small size of the MBC and MBN. Microbial N as well as transferred C and N into the microbial biomass proved too variable to draw meaningful conclusions.

Residue-C in SOM Fractions

Mineral-N inputs lead to a faster formation of recalcitrant material (Ågren et al. 2001). A faster decomposition at early stages of the incubation in the mineral-N input treatment led to a greater movement of residue-C to the more decomposed and stable fractions at the end of 90 days. Nitrogen input has been shown to help retain a significantly higher percentage of added residue and to increase structural development and aggregation of soil and residue (Lueken et al.1962). In the humin fraction, shown to be the most stable (Bird et al. 2002), a small but significantly higher amount of residue-C is recovered in the treatment with mineral-N input in the present experiment. Small differences in decomposition with mineral-N input shown in the short term can add up to large differences in the long-term storage of SOM (Ågren et al. 2001). With continuous mineral-N and residue additions, this small difference in humin N may be perpetuated, and becomes more significant with time.

More total humin C is found with mineral-N + residue amended together than with residue amended alone (*fig. 9*), suggesting that mineral-N may promote greater C sequestration in this stable pool, but this difference in total C is not statistically significant after a 90-day incubation. However, the increase in new residue-C incorporated into the humin with mineral-N input shows

a shift in the equilibrium of the stable humin fraction toward new C accumulation, indicating potential long-term changes in soil C storage.

Mineral-N may also change the transformation of C out of the humin pool. Neff et al. (2002) also found no significant net change of soil C content in a chronic N input experiment in a natural ecosystem, but they observed that mineral-N input changed C cycling among the various stable and dynamic pools. My data show a net gain of transferred plus new humin C from all treatments. Differences in total C between treatments are not significant. Thus, while measurements of total C pools were not sensitive to treatment effects on C transformation after 90 days, new C accumulation did show a difference. Bulk C assessments of changes in SOM fractions in short-term experiments therefore did not capture the subtle shift in C dynamics. Using stable isotopes, changes in new C input or losses of old C from stable C pools can be determined with greater sensitivity.

Mineral-N vs. residue-N in SOM fractions

The greatest mineral-N recovery is in the inorganic N fraction (*fig. 10*). For the mineral-N alone treatment, this is not surprising, since the absence of a C-source limits transformation of the N into more stable forms. Less, but not significantly less mineral-N is recovered in this fraction with a C-source. Under field conditions, the inorganic-N pool would be subject to leaching and lost. The greater recovery of mineral-N in the humin and POM fractions shows a larger movement of mineral-N out of the inorganic-N pool and into SOM fractions when a C-source is present. Mineral-N that moved into the POM and >500 μm fractions (*fig. 10*) may be attributed to fungal growth in these fractions that assimilated some of the added mineral-N.

A greater percentage of residue-N transformed into the fulvic acid, humic acid and humin pools than mineral-N indicating a preference for residue-N assimilation into SOM. This is in contrast to our hypothesis that mineral-N is the primary contributor to SOM formation. The mechanism for why residue-N would be more likely to contribute to stable SOM may be that mineral-N can only become a part of SOM through being incorporated into recalcitrant compounds by decomposers. For example, when lignin incorporates N and undergoes other condensation reactions in the first step of hummification (Nömmik and Vahtras 1982), or when microbes assimilate N to form recalcitrant products. Meanwhile, residue-N is already a part of organic matter when it is added to soil, it need only be stabilized as resistant SOM. Additionally, N-containing compounds can react with aromatic substances in soil to become humic compounds (Berg and Matzner 1997).

No preference for mineral-N, but residue promotes mineral-N sequestration

A trend of less residue-N is recovered in humic fractions when mineral-N is added, but significantly more mineral-N is recovered in the humin fraction when residues are added (*fig. 11*), suggesting that mineral-N is replacing some of the residue-N that is sequestered. But, there is a net benefit to N sequestration when residue and mineral-N inputs are added together. Residue and mineral-N together result in the recovery of more added-N in the humin than if either was amended alone. Residue promotes the assimilation of mineral-N into the stable humin fraction (*fig. 11*) at the cost of a significant but small amount of residue-N not being transformed into this fraction.

Since residue-N, once mineralized, should be indistinguishable from N added in mineral form, this small difference may be attributed to microorganisms showing no preference in uptake at

this stage; however, the added C-source results in greater transformation into humin overall. In this incubation, 6.5 times more mineral-N than residue-N was added in order to mimic amendment additions typical in the field. When normalized for this difference in N added, more residue-N is transformed into humin than mineral-N when both are added together (*fig.12*). Therefore, there was no preference for mineral-N to be sequestered in stable SOM and residue addition did not promote the transformation of mineral-N into humin.

Azam et al. (1985) found that plants took up less fertilizer-N when legume residues were added, and more fertilizer-N was retained in the soil and transformed into microbial biomass and humus components. Vanlauwe et al. (2001a) proposed to combine the addition of organic matter and fertilizer for its possible positive interactions and added benefits such as a delay in fertilizer-N loss to deeper layers, greater recovery of fertilizer-N in subsequent crops. Thus this may be a common phenomenon where mineral-N and residue additions to soil together enrich the soil more than adding one component alone. Besides the N benefits, organic matter and mineral-N additions together improve soil-water conditions beyond what either amendment can accomplish alone (Vanlauwe et al. 2001b).

Conclusions

Differences in the transformation of labeled microbial biomass and soil respiration were changed by added mineral-N. Carbon availability controlled the effect of mineral-N on increasing C-mineralization, with low C:N residue most affected by mineral-N input. Residue-C and N are assumed to be coupled as they pass through the decomposition pathway into soil organic matter. However, significantly more new residue-C was transformed into humin-C with mineral-N input, indicating that mineral-N alters residue decomposition and SOM formation resulting in the accumulation of new C in stable SOM. More mineral-N was transformed into humin-N with residue addition than when mineral-N was added alone, but less residue-N became humin-N in the presence of mineral-N. A possible decoupling through the microbial biomass may be initiated by mineral-N input. Microbes will take up whatever N and C is most readily available to them, even if the two nutrients come from different sources. The influence of mineral-N on ecosystem C dynamics is of great importance, due the global increase in reactive N from the burning of fossil fuels and agricultural fertilizers. Understanding how mineral-N affects decomposition and subsequent stable SOM formation in ecosystems could lead to better management strategies to optimize C sequestration in soil. This would benefit both the mitigation of global climate change and plant productivity.

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