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Metabolism of Labeled Organic Nitrogen in Soil: Regulation by Inorganic Nitrogen

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ABSTRACT

Regulation of organic N metabolism by inorganic N availability was investigated in short-term laboratory incubations of soil. A ^{14}C-, ^{15}N-labeled organic N substrate was produced by growing *Pseudomonas stutzeri* in labeled media and isolating a cytoplasmic fraction. This was added to soils that had been preincubated with glucose or glucose plus NH_4^+ to induce conditions of N deficiency or sufficiency. Regulation by inorganic N was indicated by stimulated proteolytic enzyme activity and greater initial rates of cytoplasmic ^{14}C mineralization in N deficient soils. However, effects of N deficiency on ^{14}C mineralization persisted for no more than 24 h. Preinduced N deficiency significantly decreased the extent of ^{15}N mineralized from cytoplasmic N. Mineralization of ^{14}C from leucine added to soil was similarly affected by N availability, yet ^{14}C-glutamate mineralization was apparently unaffected. In another experiment labeled cytoplasm was added simultaneously with or without a larger quantity of glucose. The glucose caused virtually complete assimilation of ^{15}N but had no effect on apparent assimilation of ^{14}C. Thus, there was no relationship between ^{15}N assimilation and ^{14}C assimilation, suggesting that the C and N contained in organic N are processed separately by soil microbes. Inorganic N availability may have short-term effects on metabolism of C in organic N but long-lasting effects appear to be minimal.

THE REGULATORY function of inorganic N (N_i) in the metabolism of organic C and organic N (N_o) in soil systems is not clear. Where N_i additions have stimulated C mineralization (CO_2 evolution) from organic materials this probably can be attributed to continuous, extreme N deficiency limiting microbial growth (Jenkinson et al., 1985). More frequently N_i amendment has inconsistent effects (Johnson et al., 1980; Shields et al., 1974) or inhibits C mineralization (Kowalenko et al., 1978; De Jong et al., 1974). In many of these studies it is not possible to assess the overall N and C (energy) availability in the unamended soils; this is likely to influence the relative response to N_i amendment. In addition to uncertainty about N_i effects on the rate of C mineralization, there is the question of N_i effects on the extent of C assimilation and mineralization, and therefore the significance of N availability in determining soil organic matter retention (Leuken et al., 1962; Pinck et al., 1950; Turk and Millar, 1936).

With regard to metabolism of N_o, pure culture results suggest that regulation by N_i, specifically NH_4^+, will be significant. In the fungi and bacteria that have been characterized, enzymes for utilization of C–N compounds tend to be regulated by both C and N availability (Marzluf, 1981; Payne, 1980; North, 1982). Yet, the growing fundamental understanding of regulation of amino acid and protein utilization obtained in pure cultures is difficult to extrapolate to soil systems. In part, this is because regulatory mechanisms and the specific effects of NH_4^+ vary among organisms. Also, N_o utilization requires distinct enzymatic steps for extracellular hydrolysis, uptake, deamination, and intracellular catabolism, each of which could be regulated differently. Although proteolytic enzyme activity and degradation of N_o have been studied in soil (Ladd, 1978), the possible significance of N_i regulation apparently has not been considered.

Some simulation models of C–N cycling in soils have accounted for the possibility of regulation by N_i and other nutrients by including demand or deficiency factors, related to nutrient availability, in the substrate decomposition functions (Bosatta and Berendse, 1984; Van Veen et al., 1981). The latter authors specifically noted that it is unclear how regulation of extracellular polymer metabolism should be described mechanistically. In conceptual models (McGill and Cole, 1981; Hunt et al., 1983) it has been proposed that soil nutrients covalently bonded with C (C–N) are metabolized to provide energy whereas ester bonded nutrients (C–O–P or C–O–S) are metabolized to provide the

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nutrient. If this is true, metabolism of C–N compounds should be regulated primarily by C availability and not N_i. Yet, this is inconsistent with pure culture studies as discussed above.

We have used ^{14}C-, ^{15}N-labeled bacterial cytoplasm and ^{14}C-labeled amino acids to determine how the availability of N_i affects the metabolism of easily degraded sources of N_o.

MATERIALS AND METHODS

Soils

Two soils were used in this study. An Argonaut silt loam (Mollic Haploxeralfs) was collected from the oak-grass savannah on the Sierra Nevada Foothill Range Stn., Univ. of California. This soil has a pH of 5.9, and organic C and N contents of 31 g C kg^{-1} and 2.2 g N kg^{-1}. The second soil was a Holland silt loam (Ultic Haploxeralfs) collected from a mixed conifer forest on the Blodgett Exp. For., Univ. of California. This soil has a pH of 5.6 and organic C and N contents of 40 g C kg^{-1} and 2 g N kg^{-1}.

Production of Labeled Organic N

Pseudomonas stutzeri was grown aerobically in media which included (per liter): 0.2 g yeast extract, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.68 g KH_2PO_4, 1.14 g $K_2HPO_4 \cdot 3H_2O$, 0.10 g NH_4Cl, 2 g glucose, 5 mg Na_2EDTA, 5 mg $FeSO_4 \cdot 7H_2O$, 1.5 mg $MnSO_4 \cdot H_2O$, 0.1 mg $CuSO_4 \cdot 5H_2O$, 0.2 mg $CoCl_2 \cdot 6H_2O$, 0.18 mg $Na_2B_4O_7 \cdot 10H_2O$, and 0.58 mg $Na_2MoO_4 \cdot 2H_2O$. For labeled batches, uniformly labeled ^{14}C D-glucose, 75 $\mu Ci\ l^{-1}$, was used and NH_4Cl was replaced with 237.7 mg $(NH_4)_2SO_4$ enriched with ^{15}N.

Cells were harvested in early stationary phase by centrifugation (4 °C, 10 000 g). These were washed repeatedly in pH 7 phosphate buffer, resuspended in water, then disrupted by sonication. A fraction consisting primarily of cell walls was removed by centrifugation. The supernatant was decanted, and acidified to pH 3 with HCl. This precipitated cytoplasmic protein and presumably other cytoplasmic polymers. Soluble components were removed from this precipitate by repeated centrifugation and washing in dilute HCl. The precipitated N_o was resolubilized by neutralizing with KOH, then frozen for storage. This material had a C/N ratio of approximately 4/1. The batch used for experiment I had an atomic % ^{15}N of 6.305; for Experiment II and IV this was 17.647.

Experiment I

Argonaut soil (35.1 g dry wt) was added to 945 mL Mason jars. This was amended with glucose, glucose plus NH_4Cl or with nothing. There were four replicates of each of these three treatments. The concentration of glucose was 1.92 mg C g $soil^{-1}$; NH_4Cl was 0.19 mg N g^{-1} soil. The final moisture content of all treatments was 0.28 g H_2O g^{-1} dry soil (0.08 MPa). The jars were sealed with covers fitted with a serum stopper for gas sampling. Unamended initial soil samples were analyzed for N_i (NH_4^+ and NO_3^-). Periodically gas samples from the jars were analyzed for CO_2. When CO_2 concentration exceeded 2% by volume the covers were removed briefly to replenish the O_2 supply. Samples were incubated at 24 °C in the dark.

After 7 d, a subsample (5 g) was removed for N_i analysis. The remaining soil was then amended with 2 mL of labeled N_o solution containing 26.9 μg N and 98.4 μg C per g dry soil. Periodically during the next 4 d headspace gas samples were collected by syringe for measurement of total and ^{14}C-CO_2. On day 11, subsamples (5 g) were removed for measurement of total N_i.

Experiment II

This was similar in design to experiment I with exceptions described below. Holland soil (15.3 g dry wt) was added to 473-mL Mason jars. All flasks received 1.96 mg glucose-C g^{-1} soil. Half of the flasks received 0.196 mg NH_4Cl-N g^{-1} soil. There were eight replications of each treatment. Moisture content after amendment was 0.31 g H_2O g^{-1} dry soil (0.03 MPa). The preincubation period lasted for 6 d. At this time four replicates were used for N_i measurement; the remaining jars were amended with labeled N_o containing 58.2 μg N and 232.7 μg C g^{-1} soil and were incubated for 4 additional days.

Experiment III

Argonaut soil (31 g dry wt.) was placed in 945-mL Mason jars and amended with 1.9 mg glucose-C g^{-1}, with 0.19 mg NH_4Cl-N g^{-1} plus glucose, or left unamended. The final gravimetric moisture content was 0.28 g g^{-1} dry soil (0.08 MPa). There were three replications per treatment. Jars were incubated as above. Periodically subsamples were removed for measurement of proteolytic activity as described below.

Experiment IV

Argonaut soil (14.8 g dry wt) was incubated in 473-mL Mason jars. Half the samples were amended with 2.03 mg glucose C g^{-1} soil and all received, at the same time, labeled cytoplasm at a rate of 0.11 mg C g^{-1} and 0.027 mg N g^{-1}. Soils were incubated as above. There were four replicates per treatment. Total CO_2, $^{14}CO_2$, N_i, and $^{15}N_i$ were measured at 228 h. Initial N_i was also determined.

Analytical Procedures

Proteolytic activity was measured by determining the rate of production of Folin reagent-reactive compounds from casein, following the procedure of Ladd and Butler (1972). Moist soil (1 g) was mixed with 5 mL of TRIS buffer (pH 8.1, 0.1 *M*) containing 50 mg isoelectric casein. This was incubated in a shaking water bath at 45 °C for 2.5 h. The reaction was terminated with 2 mL of 17.5% (wt/wt) trichloroacetic acid (TCA). After centrifugation, 2 mL of supernatant was mixed with 3 mL 2.8 *M* Na_2CO_3 and 0.33 mL Folin reagent. After 30 min absorbance was read at 700 nm. Tyrosine in an equivalent solution of TRIS buffer and TCA was used as the standard. Controls for each treatment were incubated with casein omitted. Both this value and the value for a control with soil omitted were subtracted from the appropriate sample values.

Inorganic N was extracted by shaking soil for 1 h with 2 *M* KCl, 10 mL g^{-1} soil. Ammonium and nitrate in filtered extracts were measured by flow injection analysis (Lachat Quikchem, Mequon, WI). When ^{15}N was to be measured an aliquot of the extract was steam distilled (Keeney and Nelson, 1982). The still was washed with 95% ethanol and 0.04 *M* acetic acid between samples. Distillate was collected in excess H_2SO_4 and evaporated at 90 °C in an NH_4^+-free oven. Mass spectrometer analyses were performed by Isotope Services Inc. (Los Alamos, NM).

Total N and ^{15}N in the labeled substrate were measured by conventional Kjeldahl methods (Bremner and Mulvaney, 1982), followed by distillation and analysis as above. The substrate contained no detectable free NH_4^+ or NO_3^- prior to digestion.

Total C and ^{14}C in the substrate was measured by wet oxidation and diffusion (Snyder and Trofymow, 1984). Total C was measured by titration and ^{14}C was measured by mixing an aliquot of the C trapped in base with 10 mL Scintiverse 2 (Fisher Scientific, Pittsburgh, PA).

Table 1. Total C mineralized (CO_2 evolved) during preincubation and after organic N addition to Argonaut and Holland soils with different amendments.†

Experiment	Initial amendment	Total C mineralized	
		Preincubation	After N_o
	g kg^{-1} soil	g C·kg^{-1} soil	
I Argonaut	none	0.17a*	0.16a
	1.92 glucose C	1.12b	0.32b
	1.92 glucose C, +0.19 NH_4^+–N	1.04c	0.23c
II Holland	1.96 glucose C	1.10a	0.24a
	1.96 glucose C, +0.20 NH_4^+–N	1.03b	0.19b

* Values within the same column and experiment followed by the same letter are not significantly different at the 0.05 level by pair-wise *t*-tests.
† Preincubation was day 0 to 7 for experiment I; day 0 to 6 for experiment II. Second interval was day 7 to 11 for experiment I; day 6 to 10 for experiment II.

Total CO_2 evolved during incubation was measured by gas chromatography on a 0.5 mL subsample of the headspace of incubation jars. The gas chromatograph was a Varian 91-P (Varian Associates, Sunnyvale, CA) with a Porapak QS column (Supelco, Bellefonte, PA) at 70 °C and a thermal conductivity detector at 105 °C. The carrier gas was He at a flow rate of 30 mL min^{-1}. For determination of $^{14}CO_2$, a 20 mL headspace sample was injected into an evacuated scintillation vial containing 1 mL of 0.5 *M* NaOH and sealed with a serum stopper. After at least 8 h the vial was opened, 10 mL of Scintiverse 2 was added, and ^{14}C was measured as above.

Experiment V

To further define the controls on N_o mineralization, amino acids were added to the Argonaut soil as a single known N_o source. Glucose (2 μg C g^{-1} soil) or $(NH_4)_2SO_4$ (100 μg N g^{-1} soil) were added to the soil, and incubated for 7 d at approximately 0.20 g H_2O g^{-1} soil (0.25 MPa) to create N deficiency or sufficiency, respectively. The ^{14}C-glutamate or leucine was added to 7.5 g dry weight soil at a rate of 500 μg C g^{-1} soil. This amendment brought the soil to a moisture content of 0.30 g H_2O g^{-1} soil (0.06 MPa). Soil was incubated in 473 mL Mason jars with gas-tight lids fitted with septa for gas sampling. To minimize soil drying during the assay, a beaker of water was placed in each Mason jar. Soil was incubated and preincubated at 25 °C. The jars were sampled (1 mL) for CO_2 content with a Varian 90-P gas chromatograph with detector and column conditions as described above, except that the column was operated at 35 °C. Determination of $^{14}CO_2$ was as described previously except that the scintillant was Safety-Solve (Research Products Int., Mt. Prospect, IL.).

For determination of N_i and extractable ^{14}C, 50 mL 2 *M* KCl was added to the soil, shaken for 1 h, and filtered (Whatman no. 1). The extract was analyzed for NH_4^+ and NO_2^- + NO_3^- colorimetrically. Extracted ^{14}C was measured by placing a 1 mL aliquot into a 20 mL scintillation vial containing 10 mL of scintillation cocktail.

RESULTS AND DISCUSSION

Preincubation

The intent of treating soil with glucose or glucose plus NH_4^+ was to establish N deficiency or sufficiency prior to incubating with labeled N_o. Observations during the preincubation in experiments I and II are presented primarily to define soil conditions when N_o was added (Tables 1, 2 and 3). During preincubation N_i was reduced to low concentrations in soils amended with glucose only. These results also are of interest with regard to the effect of N_i on metabolism of soil and glucose C. In experiments I and II, total C mineralized was slightly, but consistently and significantly, increased in N deficient (glucose alone) compared to sufficient treatments (NH_4^+ plus glucose) (Table 1). This NH_4^+ effect on unlabeled CO_2 evolution was also observed after N_o was added (Table 1). In these soils, under these conditions, the glucose added completely disappeared in 3 d or less and consumption was not slower with added NH_4^+ (data not shown). Therefore, the differences in CO_2 evolution shown here are not the result of NH_4^+ inhibition of glucose degradation but are due to increased assimilation of C into biomass or microbial products.

Table 2. Inorganic N during incubation of Argonaut soil with or without glucose, ammonium and organic N (experiment I).†

Amendment	N source	Inorganic N		
		Day 0	Day 7	Day 11
		mg N kg^{-1} soil		
None	Soil N	57.4a*	63.6a	65.4a
	Labeled N	—	—	9.1b
Glucose	Soil N	57.4a	5.3b	9.8b
	Labeled N	—	—	4.0c
Glucose +NH_4^+	Soil N	248.4b	94.3c	92.1d
	Labeled N	—	—	11.6e

* Values within a column followed by the same letter are not significantly different at the 0.05 level by pair-wise *t*-tests.
† 26.9 μg N_o-N added.

Proteolytic Enzyme Activity

Proteinase activity, as measured by short-term unlabeled casein hydrolysis in experiment III, was affected by glucose and NH_4^+ additions (Fig. 1). Proteinase activity was approximately constant during incubation of unamended soil. When glucose plus NH_4^+ was added, significantly increased activity was observed on day 7; before and after this time there was no significant difference from the control. This is consistent with an earlier report (Ladd and Paul, 1973). When N deficiency was induced by adding only glucose, proteinase activity was greater than both the control and the glucose plus NH_4^+ treatment on days 4 through 11.

These results indicate that proteolytic activity in soil is regulated by N_i availability. It is not possible to precisely define the mechanism of regulation. This could be at the cellular level: regulation of extracellular hydrolytic enzyme synthesis and soluble N uptake systems. Also, the N deficient vs. N sufficient incubation conditions probably would induce qualitative and quantitative differences in the soil microbial populations, thereby influencing total soil proteolytic activity. Indirect effects of N_i on enzyme stability and turnover are also possible.

Enzyme assays of this nature cannot necessarily be extrapolated to the actual mineralization of C and N in N_o substrates. Therefore, at times corresponding to the activity peaks in Fig. 1, labeled N_o was added to the soil.

Table 3. Inorganic N during incubation of Holland soil (experiment II).†

Amendment	N source	Inorganic N Day 0	Day 6	Day 10
		mg N kg^{-1} dry soil		
Glucose	Soil N	44.4a*	3.8a	4.4a
	Labeled N	—	—	13.2b
Glucose +NH_4^+	Soil N	240.5b	112.9b	107.5c
	Labeled N	—	—	43.2d

* Values in the same column followed by the same letter are not significantly different at 0.05 level by pair-wise *t*-tests.
† Glucose plus or minus NH_4^+ was added on day 0, labeled cytoplasm on day 6 (58.2 mg N_o−N added kg^{-1}).

Table 4. Carbon mineralization following addition of labeled organic N to Holland soil preincubated for 6 d with glucose and with or without NH_4^+ (experiment II).†

Treatment	^{14}C recovered as CO_2 after: 4 h	9 h	24 h	48 h	75 h	99 h
	%					
+NH_4^+	1.2a*	3.1a	12.6a	20.0a	23.4a	29.2a
−NH_4^+	1.4b	3.6b	10.1b	17.9a	22.2a	25.1a

* Values in same column followed by same letter are not significantly different at 0.05 level by *t*-tests.
† N_o added at the rate of 58.2 mg N kg^{-1} dry soil.

Mineralization of Labeled N_o

Preinduced N deficiency increased the initial rate of mineralization of C from the labeled N_o substrate. This was indicated by greater quantities of $^{14}CO_2$ evolution in Argonaut and Holland soils previously treated to induce N deficiency (Fig. 2 and Table 4, respectively). The effect in the Holland soil was less pronounced but statistically significant. These differences were observed only during a period of 24 h or less after N_o was added. Effects of N_i addition cannot be attributed to soil acidity induced by nitrification of added NH_4^+ since variation in pH among all treatments was less than 0.1 unit after the preincubation period (data not shown). These observations support the hypothesis that availability of N_i regulates soil metabolism of N_o.

On the other hand, induced N deficiency did not have a long-lasting effect on mineralization of the C contained in N_o. In experiment I (Fig. 2), 98 h after N_o was added, 36% of the ^{14}C added was evolved as CO_2 in the unamended and in the glucose plus NH_4^+ treatments, and 37% in the glucose only treatment. In experiment II (Table 4), after 99 h, 24% was mineralized in the glucose plus NH_4^+ treatment, 25% with glucose only.

The difference in mineralization between the two soils might be explained by a difference in their capacity to adsorb N_o. This unmeasured potential for adsorption also makes it difficult to estimate the fraction of N_o metabolized which was assimilated into biomass.

Nitrogen deficiency affected total C mineralization differently than it affected labeled C mineralization. While N deficiency caused an initial increase, but no long-lasting effect, on labeled C evolution, it resulted in increased total C evolution throughout the experimental period (Table 1). Unlabeled CO_2 is presumably derived from soil organic matter and from the metabolic turnover of recently added glucose. The results suggest that N regulation of these C sources may be longer-lived, and therefore of greater significance, than N availability effects on N_o metabolism. Organic N itself had no significant effect on unlabeled C mineralization from N deficient soil, as was determined by incubating the plus glucose minus NH_4^+ treatments with and without N_o (data not shown).

Nitrogen deficiency had large effects on mineralization of cytoplasmic ^{15}N, even though the glucose was depleted well before the labeled N_o was added. In both experiments, approximately three times more of the labeled N was mineralized in the N sufficient treatments compared to the N deficient (glucose only) treatments (Tables 2 and 3). This residual effect of the preincubation on N mineralization might be associated with microbial storage of C reserves or with induced changes in biomass C/N. Significantly less ^{15}N was mineralized in the previously unamended soil than in the soil with glucose plus NH_4^+ (Table 2). Since the

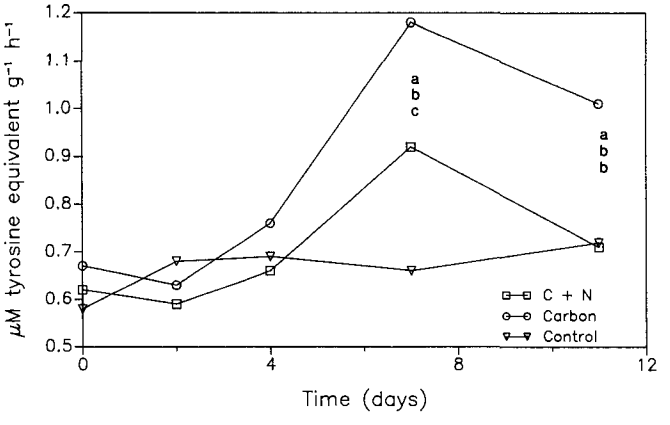

Fig. 1. Proteinase activity, as measured by casein hydrolysis, in Argonaut soil incubated with no amendment, with glucose only, or with glucose plus NH_4^+ (experiment III). Results of statistical analysis are presented in the text. Letters indicate statistically significant differences, at sampling times when they exist, by Duncan's multiple range test ($P < 0.05$).

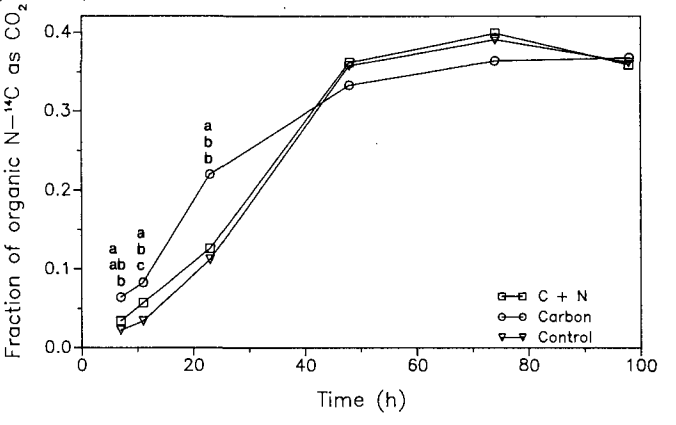

Fig. 2. Mineralization of C from cytoplasmic constituents, as measured by evolution of $^{14}CO_2$ from Argonaut soil preincubated with no amendment, with glucose only, or with glucose plus NH_4^+ (experiment I). Letters indicate statistically significant differences, at sampling times when they exist, by Duncan's multiple range test ($P < 0.05$).

latter had higher concentrations of N_i, and six times more NH_4^+, this might be attributed to preferential assimilation of NH_4^+-N over N_o.

The fraction of N_o-N mineralized was higher in experiment II than in experiment I, while the opposite was true for N_o-C. This argues against adsorption of intact N polymers or amino acids as an explanation for lower C mineralization in experiment II. Approximately twice as much N_o-N was added in experiment II and there was perhaps a limited potential for N assimilation under these conditions. Yet, the lack of any apparent correlation between C and N assimilation from the same substrate was interesting and this was investigated further in experiment IV.

Table 5. Mineralization of C and N from labeled organic N added to Argonaut soil simultaneously with or without glucose and incubated for 9.5 d (experiment IV).†

Treatment	Initial N_i	Final N_i		C mineralized	
		Total	^{15}N	Total	^{14}C
	mg N kg^{-1} soil				
Glucose	31	4	<4‡	1846	45
No glucose	31	61	17	442	46

† Organic N added at the rate of 110 mg C kg^{-1} and 27 mg N kg^{-1}; glucose added at the rate of 2.03 g kg^{-1}.

‡ Because of the small quantity of total N_i it was not possible to obtain reliable values for ^{15}N mineralized.

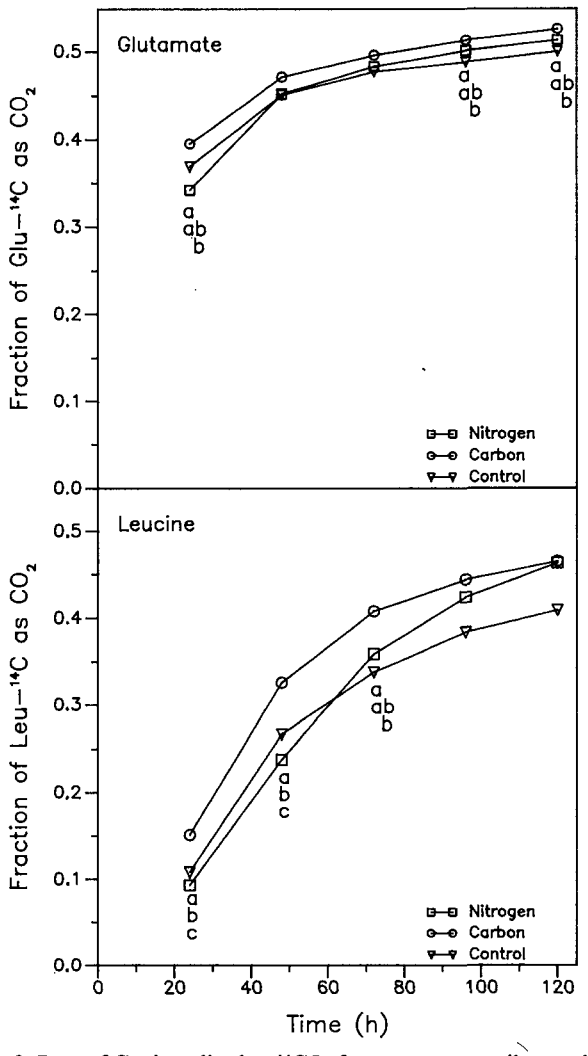

Fig. 3. Rate of C mineralized as $^{14}CO_2$ from Argonaut soil amended with labeled glutamate or leucine. Soil was preincubated with no amendment, with glucose, or with NH_4^+ (experiment V). Letters indicate statistically significant differences, at sampling times when they exist, by Duncan's multiple range test ($P < 0.05$).

In experiment IV a relatively small quantity of labeled N_o was added simultaneously with or without a larger amount of glucose. After 9.5 d, no detectable ^{15}N was mineralized with glucose, but 62% of the ^{15}N was mineralized without glucose (Table 5). Yet there was no significant difference in ^{14}C mineralization from the cytoplasmic material; 41% with glucose, 42% without (Table 5). Therefore in this case, as in the previous experiments, there was no apparent correlation between C assimilation and N assimilation from N_o. There was a slight delay in ^{14}C mineralization, lasting 2 to 3 d, when glucose was added (data not shown).

Amino Acids

Glutamate or leucine were added to the Argonaut soil as single defined organic N sources (experiment V). After 3 d incubation, less than 2% of the added glutamate-^{14}C, and 2 to 7% of the leucine-^{14}C, remained in soluble, extractable forms, indicating virtually complete consumption of the initial substrate. Preinduced differences in N availability had no significant effect on the rate of mineralization of glutamate-C (Fig. 3A). In contrast to glutamate, the initial rate of leucine-C mineralization was affected by soil N_i levels (Fig. 3B). In this case, glucose-induced N deficiency initially increased ^{14}C mineralization relative to the N sufficient treatment. However, this effect did not persist and at the end of the 5-d incubation there was no discernable difference in leucine-C mineralized. Thus, the results with leucine, but not with glutamate, are consistent with those for the cytoplasmic constituents.

CONCLUSIONS

We had expected that in N deficient soils some of the intact amino acids or nucleic acids from the substrate would be utilized anabolically. It should be energetically advantageous to use preformed amino acids to synthesize cell protein rather than deaminating and catabolizing all substrate amino acids, then synthesizing cell N_o molecules de novo. Anabolic utilization of intact amino acids would have been reflected in lower ^{14}C mineralization when ^{15}N mineralization was low. Yet this was not the case and there was no relationship between ^{14}C and ^{15}N mineralization and no detectable reduction of C mineralized from N_o by N deficiency. These results suggest that the C and N in N_o are processed independently by the soil population. Apparently amino acid and other soluble N substrates are initially deaminated completely then the N and C moieties are apportioned to anabolic and catabolic pathways.

These data provide evidence that N_i availability can affect N_o metabolism in soils. This was indicated by increased casein hydrolysis, and by increased initial ^{14}C mineralization from leucine and from cytoplasmic constituents when N deficiency was induced. These

results do not reveal the mechanism for this effect; one possibility is regulation of proteolytic enzyme synthesis by N_i. However, the effect of N_i availability was transitory and had little long-term effect on mineralization or assimilation of the C contained in N_o.

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