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Leaf-cutting ant (*Atta Sexdens*) and nutrient cycling: deep soil inorganic nitrogen stocks, mineralization, and nitrification in Eastern Amazonia

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Abstract

Nest excavation and agricultural activities of the leaf-cutting ant *Atta sexdens* create complex belowground heterogeneity in secondary forests of Eastern Amazonia. We examined the effects of this heterogeneity on inorganic-N stocks, net mineralization, and net nitrification to test the hypothesis that the bulk soil of the nests has higher net rates of mineralization and nitrification than soil that was not affected by the influences of ant nests, throughout the profile. This study was conducted in a secondary forest at Fazenda Vitoria, near Paragominas in the Eastern Brazilian Amazon, where a previous study showed that the bulk soil of ant nests had elevated NO_3^-. The results of the inorganic-N measurements were consistent with the previous study, showing elevated NO_3^- deep in the soil profile of the nests. However, neither net mineralization nor net nitrification were significantly greater at depth in the mineral soil of the nests compared to soil that was not influenced by nests ($P = 0.05$), although variability was higher in the nest soil. These results suggest that the NO_3^- may have diffused into the surrounding mineral from the N-rich organic matter buried by the ants in chambers within the deep soil.

Keywords: N cycling; Leaf-cutting ant; Net mineralization; Net nitrification; Ecosystem engineers

1. Introduction

Changes in species abundances often accompany land-use and land-cover change, which affects ecosystem function and the ability of ecosystems to recover. In Eastern Amazonia, one of the more obvious changes associated with deforestation is rapid increase of leaf-cutting ant activity (Vasconcelos and Cherrett, 1995). The leaf-cutting ant *Atta sexdens* creates nests with complex belowground heterogeneity. Cavities are dug throughout the soil profile, and occupy generally 3–7% of the soil volume, but can occupy as much as 30% of the volume (Moutinho, 1998). Cavities in the upper portions of the nest are generally used for fungus cultivation and are concentrated in the mound above the original soil surface, but can occur to a depth of 1 m. Cavities between 1 and 3 m depth are principally detritus cavities filled with the remains of leaves, fungi and ant carcasses. Throughout the nest, there are cavities that are filled with soil from other layers of the soil profile. A small portion of the cavities is empty and tunnels between cavities occupy about 2% of the belowground volume (Moutinho, 1998).

The effect of leaf-cutting ants, and the heterogeneity that they create in an otherwise relatively homogenous soil profile, on nutrient cycles is poorly understood. A recent modeling effort by Ackerman (1999) suggests that there are probably large impacts on soil C dynamics. We expect that there would be similar impacts on N cycling as these cycles are tightly coupled. The objective of this study was to further our understanding of role of leaf-cutting ants in nutrient cycling and we focused on impacts on inorganic nitrogen stocks and nitrogen cycling in the bulk soil of nests. We know from Moutinho et al. (in press) that NO_3^- concentrations are higher in the bulk soil of the nests at depths greater than 1 m than in undisturbed soil. It seems reasonable that the bulk soil in the nests would have higher amounts of available carbon and organic N, which should lead to elevated mineralization and nitrification rates in the soil profile. Therefore, we tested the hypothesis that the bulk

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soil of the nests would also have higher net rates of production of NH_4^+ and NO_3^- than undisturbed bulk soil throughout the nest profile.

2. Materials and methods

2.1. Site description

We conducted this study at Fazenda Vitória (Victory Ranch), in eastern Amazonia, near Paragominas (2° 59′ S, 47° 31′ W) in the Brazilian state of Pará. The study was conducted in a secondary forest that was regenerating naturally from a pasture that had been abandoned in 1976. Detailed site description can be found in Moutinho et al. (in press).

Nests of the leaf-cutting ant *A. sexdens* were common in this secondary forest, with an average density of 2.5 nests per hectare. Average area of the soil surface that was covered by each nest mound was 72 m^2, giving a total average coverage of 182 m^2 ha^{-1}, or approximately 2% of the soil surface (Moutinho et al., in press), Mounds were frequently 1 m or more above the original soil surface at the center of the nest.

2.2. Sample collection

For this study, three ant nests were sampled. A 0.8 m × 1.8 m pit was dug into the center of the mound to a depth of 4 m below the original surface. A control pit was dug 15 m from the edge of the mound associated with each nest, in an area with no apparent ant activity (Moutinho et al., in press). We collected samples from the walls of each pit at the following depths: 0, 10, 50, 100, 200, and 300 cm. In the case of the ant nests, these depths were sampled relative to the original soil surface. In the nests, we also sampled the soil from the mound. For each sample, the wall of the pit was scraped to expose a fresh surface. To collect the sample, we augured laterally into the wall with a bucket auger for 10 cm. All samples were collected from the bulk soil (soil that showed no visible signs of disturbance) to exclude soils from the nest chambers that were rich in organic matter. Samples were transported on ice to the laboratory where they were refrigerated until extraction and incubation.

2.3. Experimental procedures

After returning to the laboratory, all the soil samples were thoroughly mixed; coarse roots, easily visible fine roots, and coarse organic matter were removed by hand. We determined the inorganic N pool sizes by extracting NO_3-N and NH_4-N from a 15 g sub-samples of fieldmoist soil with 100 ml of 2 M KCl. Nitrate and NH_4-N analyses were done on an Alpkem (Wilsonville, Oregon, USA) autoanalyzer. Net mineralization and net nitrification were determined using the aerobic incubation procedure described by Hart et al. (1994). Details of our procedure can be found in Verchot et al. (1999). Briefly, two 15 g subsamples of field moist soil were placed in 120 ml specimen cups. One was extracted immediately, while the second was incubated for 7 days at room temperature (approx. 24 °C) and inorganic N was extracted. Net mineralization rates were determined from the difference between inorganic-N at the beginning and end of the incubation, and results were expressed on a basis of mean daily inorganic-N production. Likewise, net nitrification was determined from the difference in NO_3-N at the beginning and end of the incubation, and results were expressed in similar units.

3. Results

Ammonium concentrations were similar over the whole profile between nest and control soils, except in the top of the profile (Fig. 1). At the soil surface, NH_4^+ concentrations in the control profiles exceeded concentrations in either the mound or the old soil surface (paired *t*-test; $P = 0.008$, $P = 0.015$, respectively), which would be the appropriate comparison points in the nest profiles. On the nest, NH_4^+ concentrations were very similar in the mound and the old soil surface. Nitrate concentrations were only similar between nests and the control pits over the first meter of the soil profile (Fig. 1). At the former soil surface in the nest, NO_3^- concentrations actually exceeded concentrations in the soil surface of the control (paired *t*-test, $P = 0.041$). At depth, NO_3^- concentrations in the nests increased sharply. Although the magnitude of this increase varied greatly from nest to nest, as the large standard errors indicate, we observed this phenomenon in every nest.

The results of the net mineralization assays from control pit soils showed a typical pattern with decreasing mineralization activity with depth (Fig. 2). This pattern was repeated in the first meter of the profiles in the nests, and the nest values were not significantly different from the control values at any of these depths ($P = 0.05$). Below 1 m depth, means were not significantly different between nest and control soils, but variability increased significantly, with one nest showing similar or slightly increased net mineralization rates compared to the rest of the profile, one nest showing significant net immobilization and the third nest showing relatively high net mineralization rates at 3 m depth. Net nitrification results also follow a typical declining trend with depth in the control pits. In the nest soil, net nitrification was significantly higher at the old soil surface than at the surface of the control pits. The values for the surface of the control soils are comparable to rates determined in the mounds of the nests. Below 1 m depth, variability increased significantly, with two of the nests showing NO_3^- immobilization at each depth.

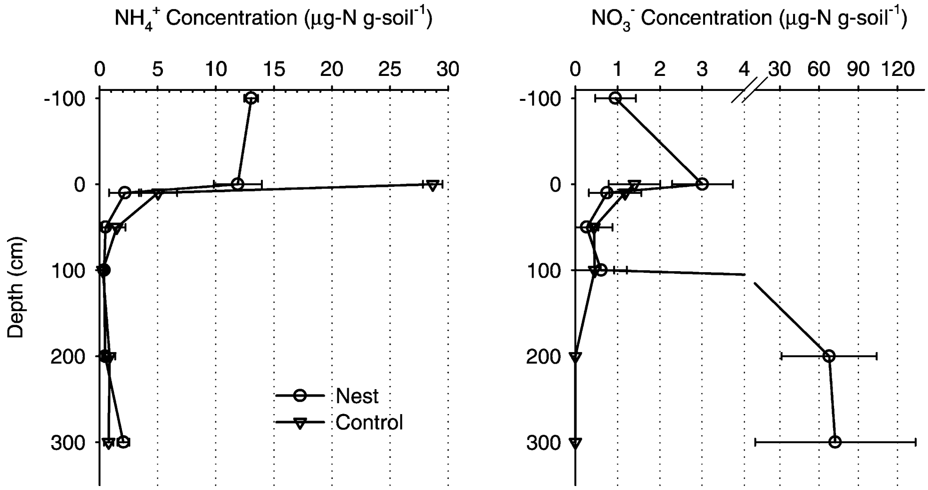

Fig. 1. Inorganic-N concentrations in the soil profiles of areas with and without ant nests.

4. Discussion

Increased leaf-cutting ant activity may have a significant impact on resource availability and ecosystem function. Leaf-cutting ants affect many soil properties due to both physical disturbance and importation of large amounts of organic matter into deeper layers of the soil profile. Physical effects include changes in bulk density, texture, and porosity (Alvarado et al., 1981). Moutinho et al. (in press) found decreased resistance to penetration in nest soils and increased root length density. Chemical effects of leaf-cutting ants are due to both the importation of nutrient-rich organic matter and to the movement of subsoil to the surface during excavation and building of the mound. This results in relatively high nutrient concentrations in the mound but the concentrations in the mound are generally lower than in the surface of undisturbed soil (Haines, 1975; Moutinho et al., in press). Moutinho et al. (in press) also found higher levels of base cations and phosphorus at depth in these nests. The color of the soil in the mounds indicates that much of it was transported by the ants from below 1 m depth (Moutinho et al., in press), and the mixing of this nutrient-poor deep soil with surface soil and organic matter yields intermediate nutrient concentrations.

We found that the major effect of the ant nests on NH_4-N was limited to the surface layers of the soil. The nests did not alter net N mineralization rates of the mineral soil samples significantly, so our hypothesis about increased N cycling within the bulk soil of the nests was not upheld. It appears that the main effect of ant nests on net N mineralization in the mineral soil was increased variability at depth, with some soils showing net immobilization below 2 m depth.

The situation for nitrate was similar between the control and nest pits in the first meter of the profile. Concentrations were slightly elevated in the nest soils at the old soil surface, and comparable in the mound to

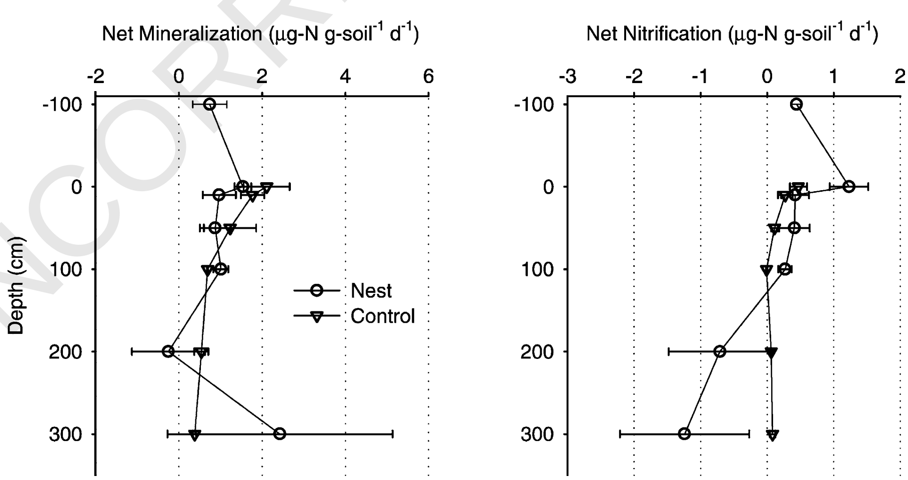

Fig. 2. Net mineralization and net nitrification rates in the soil profiles of areas with and without ant nests.

the surface soil of the control pit. Below 1 m depth, in the zone where detrital chambers were more numerous, NO_3-N concentrations soared by more than an order of magnitude, and variability increased. These increased concentrations resulted in an increased NO_3-N stock of almost 4000% in the nests. Interestingly, however, net nitrification did not increase in the bulk soil. This suggests that NO_3^- may not have been produced within the mineral soil, but rather diffused there. We speculate that net N mineralization and net nitrification may have been substantial in the detrital chambers of the nest and that the NO_3^- diffused from these chambers into the bulk soil. Since NO_3^- is much more mobile than NH_4^+, it is not surprising that high concentrations of NO_3-N are found in the bulk soil of the nests when NH_4^+ concentrations are not elevated, if the source of this NO_3^- is the refuse chambers. Moutinho et al. (in press) found that the material in the detrital chambers of the nests was relatively rich in N (2.6%) and had a low C:N ratio (7.5), suggesting that mineralization and nitrification rates could be high in these chambers. They also found that 1 M KCl-extractable potassium was elevated in the mineral soil below a depth of 1 m, with a depth profile strikingly similar to the one showed here for NO_3^-. Potassium is a relatively mobile monovalent ion and is likely the cation that diffused with the NO_3^- from the refuse chambers. Increased variability in the nitrification indices may have been associated with the position of the soil sample relative to detrital chambers, suggesting some effect on microbial processes in the bulk soil.

As far as we know, studies on the effects of leaf-cutting ants of the genus *Atta*, on soil chemical properties have looked only at nutrient concentrations and stocks (Haines, 1975, 1978; Moutinho et al., in press). This is the first study that we know of where soil microbiological dynamics have been investigated. This study shows that *Atta* species do indeed alter nutrient availability in secondary forest ecosystems of eastern Amazonia. The likely mechanism for this alteration is increased microbial activity, but this increased activity is not generalized to the bulk soil of the nests, rather it is probably limited to the fungus and detrital chambers.

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