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Tracer methods to assess nutrient uptake distribution in multistrata agroforestry systems

J. Lehmann[1, 2, *] and T. Muraoka[3]
[1] *Institute of Soil Science and Soil Geography, University of Bayreuth, 95440 Bayreuth, Germany;* [2] *Federal Research Institute for Forestry and Forest Products, Hamburg, Germany;* [3] *Centro de Energia Nuclear na Agricultura CENA, University of Sao Paulo, 13400-970 Piracicaba, Brazil (*Author for correspondence: Current address: Department of Crop and Soil Sciences, Cornell University, Ithaca NY 14853, USA, E-mail: CL273@cornell.edu)*

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Abstract

Separate assessment of nutrient uptake by individual plants in mixed cropping with trees is impossible without tracer techniques. The different ^{15}N-to-^{14}N isotope ratio of atmospheric and soil N can be used to study the contribution of biologically fixed N to the nutrition of associated trees. In most cases, the assessment of nutrient uptake distribution is an appropriate way of evaluating how to improve the transfer of biologically fixed N. Radioisotopes (e.g., ^{32}P), stable isotopes (e.g., ^{15}N) and rare elements (e.g., Sr) can be used to determine relative root activity distribution by applying the tracer to different soil depths or distances from trees. A broadcast application of the tracer instead of point application makes it possibe to calculate uptake values per unit area. The direct determination of nutrient pathways with such robust experiments offers considerable advantages for improving nutrient use efficiency and complementarity in multistrata agroforestry systems.

Introduction

Complementarity of nutrient uptake is an important goal for intercropping different trees and crops, and in order to increase nutrient use efficiency and production. On the other hand, nutrient competition may decrease crop production (Schroth et al., 2001). However, improved N nutrition and growth of a non-legume species may be achieved if it has access to biologically fixed N from an associated legume tree. Advantages or disadvantages of intercropping for plant nutrition are difficult to assess in agroforestry systems due to their spatial and/or temporal discontinuity. Multistrata agroforestry systems with perennial plants have an especially high complexity, because they may comprise several different tree species in complex spatial patterns with contrasting above and below-ground biomass distribution. Tracer techniques can help identify and also quantify spatial and temporal patterns of nutrient uptake, but they have rarely been used (Thomas et al., 1998; Kumar et al., 1999) in multistrata agroforestry systems. The use of tracers in agroforestry research has been principally limited to alley cropping systems (Haggar et al., 1993; Rowe et al., 1999). In this paper, we highlight and discuss the applicability of tracer techniques to study nutrient uptake pathways in multistrata agroforestry systems.

The use of tracers for assessing nutrient uptake by trees

Three conceptually different classes of tracers of interest for multistrata agroforestry research which are: (a) rare elements (e.g., Sr^{2+}, Rb^{+}); (b) radioisotopes (e.g., ^{32}P, ^{33}P, ^{35}S); and (c) stable isotopes using tracer applications or evaluations of natural abundance (e.g., ^{15}N, ^{34}S, ^{18}O). The handling, mode of application, analytical procedure and accuracy, with respect to environmental behavior, price and availability, differ for the three classes (Vose, 1980).

The reason for the limited use of tracers in agroforestry systems may be because of the technical difficulties involved. Most agroforestry studies have been undertaken in regions where specialized analytical equipment, needed to measure tracer concentrations, is not available. The high costs of some of the tracers and analyses also restrict their use. Other reasons include the lack of methodologies for using tracers in agroforestry research. Nevertheless, tracer studies conducted in agricultural systems (Vose, 1980; Danso et al., 1992; Zapata and Hera, 1995), and more recently in natural ecosystems (Högberg, 1997), offer a wide range of potential methods to identify nutrient pathways in multistrata agroforestry systems.

Tracer techniques use the difference in the relative abundance of a rare element or an isotope (e.g., proportion of ^{15}N in tree leaves in different treatments), measured as a concentration, isotopic mass or radioactivity. Uptake can be followed by measuring its abundance in the source (e.g., ^{15}N, in the air, soil or fertilizer) and in the plant and soil compartments. Tracers can be used to determine: biological N_2 fixation rates of tree legumes (Danso et al., 1992); nutrient use efficiency of applied inorganic fertilizer, mulch or animal manure (Hauk et al., 1994; Zapata and Hera,1995); or nutrient uptake by trees from different soil nutrient pools (Haggar et al., 1993; Frossard et al., 1999). These methods have considerable value for studying nutrient uptake in multistrata agroforestry systems, but since they are not specific to systems with perennial crops they are not discussed in detail here.

Tracer applications introduce a new dimension to agroforestry research, as they can identify the uptake of nutrients by each tree in mixed cropping systems. In a tree fallow, nitrate profiles in acid soils may indicate the depth of N uptake by the plant association (Mekonnen et al., 1997), but the uptake by individual trees in multistrata agroforestry systems can not be determined with such a technique. Using tracers, it is possible to determine the nutrient uptake distribution in agroforestry systems with many intercropped trees.

Tracer studies in multistrata agroforestry

Transfer of nutrients between associated trees

The transfer of nutrients between trees includes the utilization of N, biologically fixed by a legume tree, by an associated species, or the uptake of other nutrients (e.g., P, Zn, Cu) by species A, which were transformed into more plant available forms by species B. The transfer usually comprises two different processes: the transfer may originate directly from increased uptake by species A due to increased nutrient availability in its rhizosphere (e.g., root release of biologically fixed N; microbial P solubilization; desorption of P by organic acids by species B) or by recycling from litter return by species B. Only the transfer of biologically fixed N is discussed below.

Transfer of biologically fixed N can be determined by measuring the ^{15}N contents in a non-legume which is grown in association with a legume in comparison to a legume-free control (for mixtures of annual crops, see Chalk and Smith, 1994). Since the N fixed from the atmosphere has a lower ^{15}N content ($\delta^{15}N$ of 0‰; the $\delta^{15}N$ notation is the ratio of ^{15}N-to-^{14}N set to 0 for the atmospheric ^{15}N content and given in ‰) than the N taken up from soil (in agricultural soils a typical $\delta^{15}N$ is between 3 and 10), N_2-fixing legumes have lower biomass $\delta^{15}N$ values. If N is subsequently released from the legume to the soil, the proportion of ^{15}N available from the soil for an associated non-legume is lower. Such studies have to meet the requirements for the determination of biological N_2 fixation. Considerable difficulties have been encountered with ensuring the same soil ^{15}N-to-^{14}N uptake of legume and reference species (Witty, 1981; Danso et al., 1992), and the same is true for the uptake of non-legume-N

in the legume intercropping in comparison to the non-legume monoculture (Giller, 2001). The large volume of soil used by trees makes it almost impossible to provide a soil source with a homogeneous ^{15}N-to-^{14}N ratio through the application of ^{15}N to soil. For annual crop studies, the topsoil may be mixed after applying the isotope. This is not possible for deeper rooting plants such as trees. Even using the natural abundance method, uniform $\delta^{15}N$ values with depth are rather the exception than the rule. In central Amazonia, $\delta^{15}N$ values of 8‰ were found at the topsoil and 23‰ at 1.5 m depth under primary forest (Piccolo et al., 1996). Even lower differences between top- and subsoil may obscure the results, as the ^{15}N difference between legume-N and topsoil-N may not exceed a few permil in $\delta^{15}N$ units.

This constraint may be overcome in determinations of biological N_2 fixation by trees, if the legume and non-legume have the same vertical root activity distribution. This would translate into the requirement of the same root activity distribution for the non-legume grown in association with a legume compared to the non-legume in monoculture, which may not hold if inter-specific root competition affects root distribution. In the central Amazon, the foliar $\delta^{15}N$ values were lower in *Theobroma* monocultures without a *Pueraria* cover crop than in *Theobroma* in a multistrata agroforestry system with high abundance of *Pueraria* (J. Lehmann, unpublished data). This would indicate that more biologically fixed N was recovered by *Theobroma* in monoculture than in mixed cropping, even though there was no legume present in the monoculture. The unexpected result may be explained by the more superficial root system of the *Theobroma* monoculture, which acquired topsoil N with lower ^{15}N values, in the absence of competition from other trees and from the legume cover for soil nutrients and water. This problem may be solved with adequate control plots, which include similar mixtures of plant species and arrangements to avoid different root activity occuring in experimental plots and controls; e.g., by substituting the N_2-fixing legume with a non-N_2-fixing strain of the same plant species.

Snoek (1995), using the natural ^{15}N abundance method, calculated that 6 to 22% of the N in coffee was derived from N biologically fixed by the associated legumes *Flemingia macrophylla*, *Leucaena diversifolia* or *Desmodium intortum*. The value increased with higher N_2 fixation of the legumes themselves (20 to 52% of the N in the legume) providing a strong indication for N transfer. The comparison of coffee systems with intercropped legumes having different N_2 fixation avoids, to a certain extent, the danger of changing root systems because competition differs. The absolute values of the determined N transfer should still be viewed with caution, because the coffee in the legume-free control may have a different root distribution and therefore tracer uptake than the intercropped coffee.

The horizontal redistribution of ^{15}N, when enriched ^{15}N is applied to elevate the difference between the soil and biologically fixed N, is an additional problem. When enriched ^{15}N was applied in an agroforestry system of *Acacia saligna* intercropped with sorghum (*Sorghum bicolor*), *A. saligna* accumulated and enriched the soil underneath the tree canopy with ^{15}N (Lehmann et al., 2001a). The result was a very high estimate of transfer of biologically fixed N to the intercropped sorghum, not because the tree was diluting the soil underneath the sorghum with N having low ^{15}N enrichment, but because the tree was successfully competing for the applied ^{15}N. Different N uptake patterns were found to be responsible for a similar observation made by Viera-Vargas et al. (1995) with respect to N transfer from a herbaceous legume to an associated grass.

The same may even happen in control plots of non-fixing legume strains, when they are relying more on soil N than the N fixing legume, which acquires part of its N from the atmosphere. The non-fixing legume may compete for the applied soil ^{15}N more than the N fixing legume and decrease the ^{15}N uptake of the associated non-legume, thereby lowering the estimates of N transfer.

Injections of ^{15}N into trees may provide a more direct answer to the question of N input to soil by leguminous trees (Horwath et al., 1992), but do not give quantitative results about the transfer of biologically fixed N. Using this technique in a temperate climate alley cropping system, the fluxes of ^{15}N injected to alder trees to intercropped corn could be followed separately through either

root or shoot litter (Seiter and Horwath, 1999). This information may be used to identify the most important pathways by which a non-legume aquires legume N. However, before these methods for a direct assessment of nutrient transfer are employed, it is necessary to verify that the tree, which is presumably benefiting from the nutrient transfer, is rooting in the zone where increased nutrient availability occurs.

Nutrient uptake distribution

The assessment of nutrient uptake distributions is essential for estimating competition (George et al., 1996; Thomas et al., 1998) and the possibility of nutrient transfer between associated tree crops (Lehmann et al., 2000), as well as complementarity of nutrient acquisition and subsoil nutrient recycling (Rowe et al., 1999). Studies of nutrient uptake from different depths using tracers (Hall et al., 1953) are non-destructive alternatives to measurements of root abundance and may also be a more valid indicator of root activity than root length density. The tracer is injected at a certain depth under a tree and its foliar contents are measured after a few weeks. The same is done at a different depth for another individual of the same species with the same management. Finally, tracer enrichment in foliage after application at one depth is given as the percentage of enrichment from another (or several other) depth(s) and interpreted as root activity distribution. The high variability of the results, usually between 30 and 157%, is a disadvantage of this method (IAEA, 1975). Additionally, total uptake from different soil volumes cannot be quantified with this approach. However, comparisons of the importance of different soil depths for crop nutrition can be compared for different tree associations; management interventions (e.g., pruning *vs.* no pruning), cropping systems (monoculture *vs.* mixed cropping) or seasons (dry *vs.* wet season).

A comprehensive IAEA (1975) study of the use of P radioisotopes for determining the distances and depths of nutrient uptake by several tropical fruit trees, was not followed by further methodological work. Recently, ^{15}N has been used in alley cropping, in order to assess the depth of nutrient uptake between hedgerows of *Gliricidia sepium* and *Peltophorum dasyrrhachis* in Indonesia (Rowe et al., 1999). Problems are created by the high mobility of N compounds, and the exact area of N uptake is difficult to control. To overcome this constraint, sucrose may be applied to enhance microbial immobilization of ^{15}N (Rowe et al., 1999). Furthermore, due to the large dilution in plant and soil, the analysis of N isotopes is less sensitive than that of radioisotopes and highly enriched expensive ^{15}N substances must be used.

In a multistrata agroforestry system on clayey and acidic soil, applying the stable ^{15}N isotope to different depths yielded similar estimates for root activity distribution as did applying radioactive ^{32}P (Figure 1). Therefore, for root activity measurements, it seems to be feasible to substitute the hazardous P radioisotope by ^{15}N. In fact, the N uptake in the cited study showed a lower variability between replicates than the P uptake (Figure 1), presumably because P is very immobile and only a small soil volume can be labeled with P. Although this may be a possible advantage to exactly locate P uptake, it may be a disadvantage when it is necessary to label sufficient soil volume, and may not be compensated even by a large number of applications per tree (IAEA, 1975). Using ^{15}N offers an advantage in this respect, as a larger volume is more easily labeled due to the high mobility of nitrate, reducing the variability of the root activity measurement. However, the magnitude of N movement from the point of application should be verified by repeated soil analyses and/or with some parallel ^{32}P measurements to check the validity of the ^{15}N results. The use of ^{15}N may not work, however, under all environmental circumstances; e.g., under high leaching conditions in sandy soils. Additionally, ^{15}N may be advantageous because it showed a better correlation between old and young leaf ^{15}N contents ($r^2 = 0.682$; $\rho < 0.001$) than did ^{32}P ($r^2 = 0.432$; $\rho < 0.001$; Lehmann et al., 2001b) and hence the effect of the leaf sampling strategy was less important. The analyses of young leaves should be preferred because ^{15}N values of old leaves are generally lower. On the other hand, experimentation with the stable isotope ^{15}N does not allow repeated assessments of the root activity distribution of the same tree (e.g., in different seasons) in contrast to the radioisotope ^{32}P, which possesses a very short half life of only 14.3 days.

Rare elements, though seldom used in this

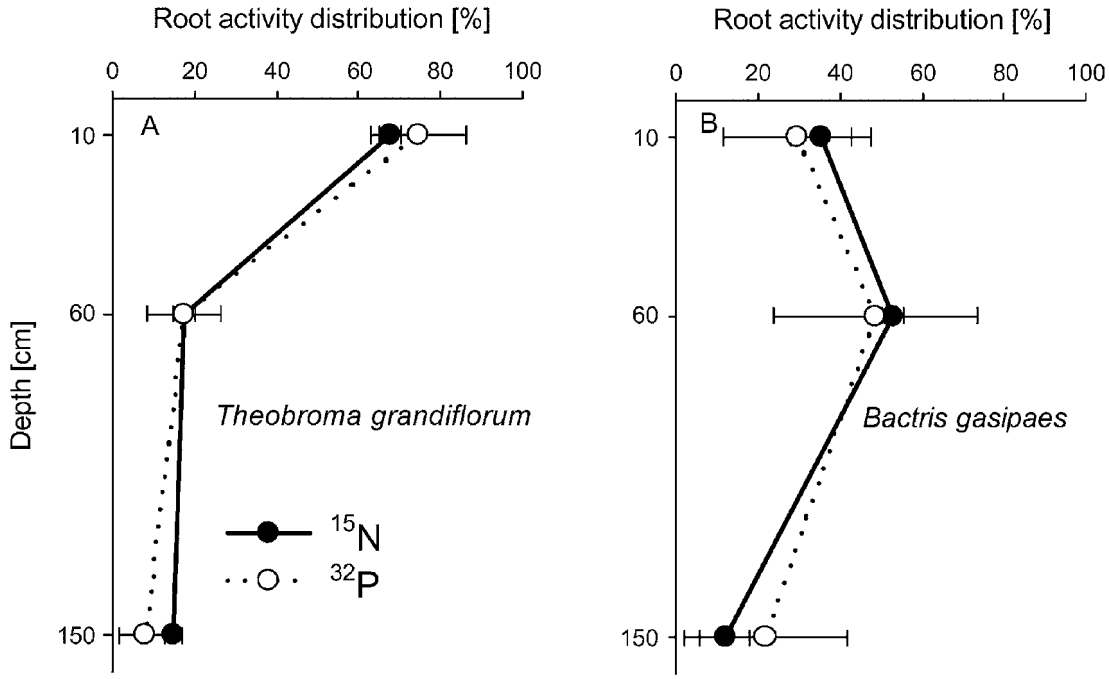

Figure 1. Root activity distribution of (A) *Theobroma grandiflorum* and (B) *Bactris gasipaes* determined by foliar isotope analyses 21 days after applying 1 mCi ^{32}P tree^{-1} or 0.1 g ^{15}Nexcess tree^{-1} at 0.1, 0.6 or 1.5 m depth in a Xanthic Ferralsol under a multi-strata agroforestry system of central Amazonia during the dry season (Lehmann et al., 2001b). (n = 3)

context (e.g., Van Rees and Comerford 1986; Lehmann et al., 1999) deserve more attention. Fox and Lipps (1964) detected a similar root activity distribution of alfalfa when determined with Sr or ^{32}P (Figure 2), suggesting that Sr is a feasible alternative to ^{32}P. Nevertheless, it must be kept in mind that rare elements may not be as rare as expected: in a tropical dryland, large amounts of exchangeable Sr were found in Ca-rich soils, making it difficult to measure a Sr tracer which was applied (D. Weigl and J. Lehmann, unpubl. data).

Tracer techniques for the determination of soil water uptake from different depths also provide an indirect indication for nutrient uptake distribution. The complementarity of the root activity of different plants can be tested by comparing the natural abundance of stable oxygen and hydrogen isotopes. The basis for this approach is the natural discrimination due to water evaporation and consequently higher abundance of the heavier isotopes near the soil surface. For example, the vertical stratification of soil water uptake between *Azadirachta indica* and intercropped millet in a

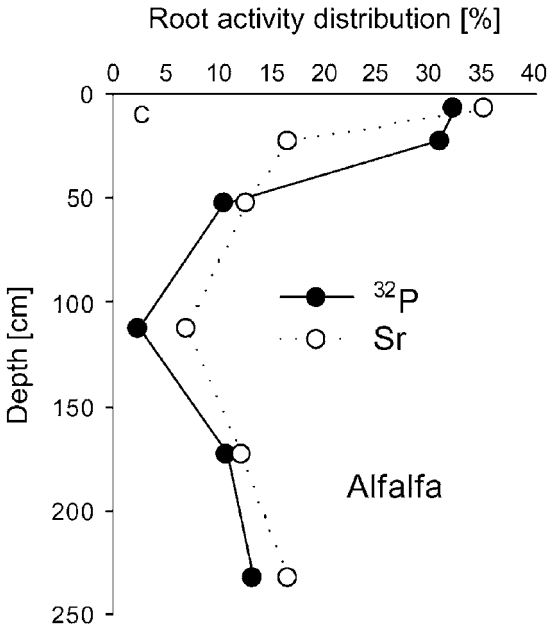

Figure 2. Root activity distribution of alfalfa (*Medicago sativa*) determined with Sr and ^{32}P in Chernozem soils of Nebraska during summer (Fox and Lipps, 1964). (n = 3)

Sahelian windbreak system (Smith et al., 1997), or between different trees in the Brazilian Cerrado, could be compared (Jackson et al., 1999).

Lateral nutrient uptake of different tree crops in multistrata agroforestry can also be studied with ^{15}N tracers, as was shown for different acacias and coconut (*Cocus nucifera*) in Côte d'Ivoire (Zakra et al., 1991) or using ^{32}P for ginger (*Zingiber officinale*) and *Ailanthus triphysa* in humid India (Thomas et al., 1998). Instead of applying a point source, lateral uptake may also quantitatively be determined by labeling whole areas within the agroforestry system. For example, it could be shown that *Theobroma grandiflorum* took up more N from areas covered with the legume *Pueraria phaseoloides* than did the associated palm *Bactris gasipaes* by applying ^{15}N enriched ammonium sulfate to three areas in a multistrata agroforestry system (Figure 3; Lehmann et al., 2000). The ^{15}N distribution will not be uniform in the soil profile, but ^{15}N will concentrate in the topsoil. This will give a bias suggesting higher activity of trees with a superficial root system. When evaluating the proportion of fertilizer or biologically fixed N taken up by different trees, this is a valid scenario, as these N sources would normally be found in the topsoil. The low proportion of root activity of *Theobroma* and *Bactris* in the area underneath the legume cover crop (generally being less than 20% of their individual N uptake) made it unlikely that the tree crops would benefit to a large extent from the N biologically fixed by the legume. Such a result suggests the importance of management interventions which increase tree root activity underneath the legume to provide the tree with more of the biologically fixed N. Direct determinations of N transfer are only justifiable if root activity measurements have shown that non-leguminous components have the potential to acquire N from an area in the agroforestry system which potentially contains biologically fixed N. The same applies to other benefits of associated trees (e.g., enrichment with plant available P).

Conclusions

Tracer techniques are potentially useful for assessing nutrient uptake distributions in multistrata agroforestry systems, and they can be ideal for studying the complementarity of nutrient uptake in space and time. Robust experiments in multistrata agroforestry systems include the comparison of nutrient uptake by a tree at two or several points in the cropping system. The tracer uptake relationship can be compared between different tree species, management (pruning *vs.* no pruning), cropping systems (monoculture *vs.* mixed cropping) or seasons (dry *vs.* wet season). Unfortunately these relationships do not give

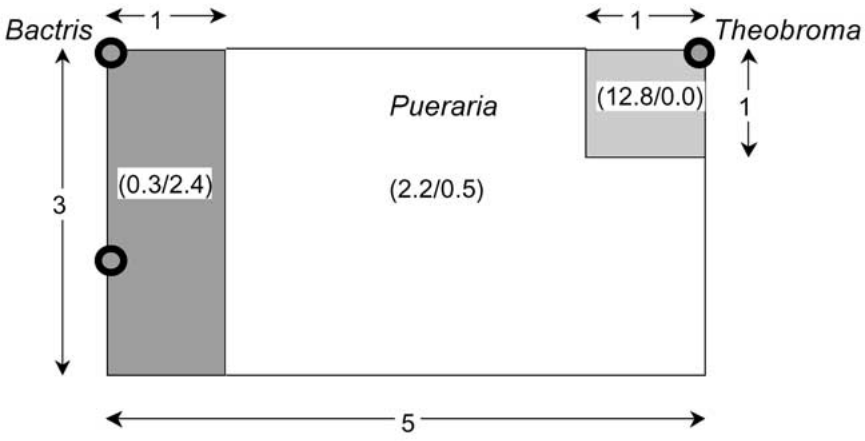

Figure 3. Total ^{15}N recovery by *Theobroma grandiflorum* and *Bactris gasipaes* after broadcast fertilizer application to three subplots under either *Theobroma* ■, *Bactris* ■, or *Pueraria phaseoloides* grown between the trees □ in a multistrata agroforestry system of the central Amazon (Lehmann et al., 2000). Values given in parenthesis are the % recovery in each sub-plot, by *T. grandiflorum* and *B. gasipaes*, repectively, of the applied 3 g ^{15}N excess ha^{-1}; numbers between arrows are distances in m; differences between relative uptake are significant at $P < 0.05$.

information on total uptake per unit area. However, the labeling of whole areas is a step towards the quantification of total nutrient uptake from different areas in the cropping system, which can be compared with other nutrient pools or pathways such as the total above ground nutrient stocks or nutrient leaching. Studies on nutrient uptake distribution should be conducted before attempting an assessment of nutrient transfer between species; e.g., of biologically fixed N. Although this paper emphasizes the need to exploit the potential of tracers to assess nutrient uptake distributions in multistrata agroforestry systems, further studies are required to develop tracer techniques which can be easily conducted and unambiguously interpreted.

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