PHOSPHORUS DYNAMICS IN THE LITTER LAYERS OF A TRADITIONAL SLASH-MULCH BEAN PRODUCTION SYSTEM IN SOUTHERN COSTA RICA: REASSESSING CONCEPTS AND METHODS IN LITTER DECOMPOSITION RESEARCH

by

ISIDOR FORREST RUDERFER

(Under the Direction of Bruce Lee Haines)

ABSTRACT

A major portion of the beans consumed in Costa Rica are produced under a traditional slash-mulch system referred to as *frijol tapado*. Research is under way to assess the possibility of improving yields and sustainability of the system through fallow enrichment with typical agroforestry tree species. Selecting appropriate mulch-providing species for use in this and other agroforestry systems requires detailed knowledge of their nutrient-release behavior during decomposition. Standard decomposition and nutrient release models are of little use in this context because (1) they assume (usually implicitly) that all litter is in direct and continuous contact with the soil solution and (2) they do not reflect the fact that nutrients flow into and out of the litter simultaneously along different paths (Berg 1988) and, as a consequence, net change in litter nutrient content does not necessarily correspond to nutrient availability in the litter layers and maybe even the soil.

INDEX WORDS: decomposition, litter, mulch, litterbag, litterbasket, phosphorus, nutrient uptake, immobilization, San Vito de Java, Coto Brus, Costa Rica, *Inga edulis*, *frijol*

tapado, slash-mulch, agroforestry, nutrient release, indigenous agriculture, nutrient dynamics, OTS-Las Cruces Biological Station, Wilson Botanical Garden, no-till, no-drill, agroecology

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DEDICATION

1) Parents
2) Tanya Cofer
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CHAPTER 1

INTRODUCTION

As recently as the early 1980s, more than half of the area dedicated to bean-production in Costa Rica was managed in the form of a traditional slash-mulch system referred to as *frijol tapado* (Alfaro 1984, in Gonzalez M. and Araya V. 1994). Research is under way to assess the possibility of improving yields and sustainability of the system through fallow enrichment with typical agroforestry tree species (Kettler 1995, Kettler 1997b, Kettler 1997a). Selecting appropriate mulch-providing species for use in this and other agroforestry systems requires detailed knowledge of their nutrient-release behavior during decomposition. I argue that standard decomposition and nutrient release models may be of little use in this context because (1) they assume (usually implicitly) that *all* litter is in direct and continuous contact with the soil solution and (2) they do not reflect the fact that nutrients flow into and out of the litter simultaneously (Berg 1988), apparently from different pools, and, as a consequence, this net change in litter nutrient content does not necessarily correspond to nutrient availability in the litter layers and maybe even the soil. Revising these models is undoubtedly necessary for analyzing the very specific agroecological context of the *frijol tapado* system with its multiple litter layers. However, these new models may also be of general ecological

applicability, providing insight into the nutrient dynamics of other contexts such as forest canopies, tropical forest floors with surface root mats, and temperate forest understory plants with roots in the litter layer.

Standard methods in litter decomposition research are designed to measure variables defined as important by the standard models. Revising the models will therefore require some revision of methods as well. Using traceable nutrient isotopes is the only way to definitively collect some of the needed data. However, because of their cost and/or regulatory complications, isotope experiments are simply not a practical alternative in many cases, especially for those doing small-scale agroforestry research in the tropics. Furthermore, the short half-life of ^{32}P limits its usefulness in such studies.

In this study, I design a variation on the multilayer litterbasket decomposition mesocosm (Blair et al. 1991) in an attempt to obtain the data needed for my proposed revised model without having to resort to using traceable isotopes. These containers I constructed had three layers separated by nylon mesh screening: (1) on top, *Inga edulis* prunings (mulch), (2) below the mulch, approximately 1 year-old decomposed *Inga edulis* prunings (litter), (3) plastic bags to collect leachate. These containers were accompanied by (1) "rain blanks" to correct for atmospheric phosphorus deposition and (2) "false mulch" blanks with a plastic mulch instead of the fresh mulch on top of the old litter. The "false mulch" blanks were needed to determine, approximately, how much

phosphorus leached from the old litter by itself in the containers with both layers.

The decomposition environment in these containers was obviously very artificial. In order to determine whether it was too artificial to be of any use, I tested my containers against the accepted and well-known artificialities of the standard litterbag decomposition environment. I hypothesized that all the artificiality added to the decomposition process by my modified litterbaskets could be attributed to one or both of two sources: (1) absence of soil in the system, and (2) the plastic-tub microenvironment. In comparing my modified litterbaskets to litterbags, the former had both sources of artificiality, and the latter, none. Therefore, I added a third treatment which I hoped would occupy an intermediate position on the artificiality spectrum because it had only one of these sources of new artificiality: the "plastic-tub microenvironment". This third treatment, which I refer to as the "Soil Surface" treatment, is identical to the Leachate-Collector treatment in all regards except one: instead of having the two plant material layers suspended over a plastic bag, they are placed on the soil surface.

This three-treatment test had two purposes: (1) determine whether decomposition occurs similarly in the multi-litter-layer leachate collector as it does in the standard litter bag, and (2) determine whether this container design makes it possible to provide bounded estimates of gross

nutrient flows into and out of fresh litter which are normally hidden by net change measurements in standard litter decomposition experiments. The three treatments and associated blanks were placed under open sky in the field on the grounds of the OTS-Las Cruces Biological Station for twenty three days beginning August 14, 1999. Each treatment had ten replicates, and these were accompanied by five "rain blanks" and four "false mulch" blanks. At the start and the end of the field exposure period, the following measurements were taken for each treatment-replicate-layer unit: (1) dry mass (DM), (2) total P (P), (3) microbial P (MP), which is calculated by subtracting bicarbonate-extracted P (BEP) from chloroform-fumigated, bicarbonate-extracted P (CFBEP). The leachate bags filled and were collected three times over the course of the experiment.

By combining the data for total P loss from the leachate collectors and fake-mulch blanks, I was able to measure a portion of the gross outflow from the fresh litter layer. My intention is to compare this measurement to the standard measurement of nutrient release from litter: change in litter nutrient content. If, in fact, nutrient release is occurring in the ways not accounted for in the standard model of decomposition, I should be able to observe a difference between these two measurements of nutrient release.

In the next chapter, I analyze the research that was the starting point for my work, the dissertation of James S. Kettler (Kettler 1995) and the publications that followed from it (Kettler 1997a, Kettler 1997b). He studied a number of aspects of *frijol tapado* (which I will describe in more detail below), including (1) its economic efficiency compared to a high-energy-input system and (2) the possibility of improving the system through the use of agroforestry trees planted specifically to provide more-consistent, higher-quality mulch for the bean crop. As part of the latter work, Kettler attempted to isolate some of the mechanisms of nutrient transfer from the decomposing mulch to crop plants. I discuss his results and asses some of the questions that still need to be addressed in order to arrive at a better understanding of nutrient dynamics in this system.

In chapter three, I assess some aspects of the conceptual model at the center of most litter decomposition work. I argue that litter decomposition research imported some theoretical machinery from agronomic research on nutrient dynamics in soil-incorporated plant residues to explain some seemingly anomalous observations. In the process, though, they inadvertently imported some assumptions which are not always met in the context of surface-litter decomposition processes. I discuss the possible implications of not meeting those assumptions for litter decomposition models, and by extension, the methods and interpretations based on those models.

In chapter four, I discuss my own attempt to take these violated assumptions seriously. I propose a method for measuring flows in the litter decomposition process which cannot be observed using the standard methods. I discuss how I will use several different decomposition environments/containers to calculate these flows. I also discuss how I will attempt to isolate some of the inevitable new artificialities introduced by the use of my new method.

In chapter five, I report on the materials and methods I used to measure the variable identified in chapter four. Chapter five documents the results of my study. In chapter seven, I discuss these results, and in eight, I make recommendations for future research.

1.1. Frijol Tapado: slash-mulch, no-till and *"no drill"*

1. Slash-and-mulch system vs. slash-and-burn.
2. Common bean, *Phaseolus vulgaris*, sometimes mixed with *Zea maiz*.
3. Low yield/area, high yield/investment.
4. Plays an important role in family-level subsistence.
5. Accounts for significant percentages of national production (~40%) and area devoted to production (~60%) (according to studies from the mid-90s).
6. How it works:
    6.1. Before the end of the rainy season (in southern Costa Rica: late November, early December) farmers identify parcels of land with a

desirable stand of fallow vegetation. They then chop paths through this vegetation roughly every 5 meters.

6.2. They hand-throw (broadcast) beans in a practiced manner into the standing vegetation.

6.3. The vegetation is chopped down on top of the beans (and rechopped into finer pieces).

6.4. The crop is left untouched until harvest after approximately three months, during which time the rainy season is supposed to end.

1.2. Terminology

<<

Mulch vs litter; vs. $O_1 \ldots O_n$; F vs. L, O etc.

plant residue, crop residue, green manure

litter, "intermediate litter" (Loma Linda terminology)

probably better to put this in the intro

>>

CHAPTER 2

J. KETTLER'S *FRIJOL TAPADO* RESEARCH

In 1992, James S. Kettler began a complex multi-year set of experiments to study the potential for improving yields and sustainability in *frijol tapado* plots through the use of trees planted to provide higher quality mulch (Kettler 1995, 1997a, 1997b). My own research was designed to build on Kettler's work and the work of others studying nutrient dynamics in *frijol tapado*. As part of introducing the goals and design of my research, I will present, below, a summary of his work and my own analysis of some of his data. Kettler addressed many important aspects of the *frijol tapado* system in his research, but here I will summarize only the work relevant to nutrient cycling.

Field plots were established using four different agroforestry species, three mixtures of those species, and one control replicating the field preparation typically used by local farmers (Kettler 1997a):

Control
1) *Frijol tapado*
Single species
2) *Calliandra calothrysus*
3) Erythrina poeppigiana
4) *Gliricidia sepium*
5) *Inga edulis*
Mixed species
6) *Inga*/*Calliandra*
7) *Inga*/*Erythrina*
8) *Inga*/*Gliricidia*

Using a randomized block design with five replicates, he measured dry mass and nutrient inputs (N and P) of mulch from the treatment trees as well as that from unplanted secondary vegetation growing in the plots ("weeds"). After the customary growing season of three months, the bean plants were harvested and the bean seed yield was measured. *Erythrina poeppigiana* suffered an insect infestation which rendered it useless as a source of mulch, and therefore it was dropped from the study.

In a concurrent study, Kettler set out to elucidate nutrient dynamics in the decomposing mulch of the same species in the more controlled conditions of a pot study (Kettler 1997b). In this study, Kettler raised beans in containers of soil with surface-applied mulches. He compared initial N and P contents of the applied mulch to N and P availability in the soil and uptake by bean plants over a 50-day period.

Using the pot study to shed light on the mechanisms behind the results of the field study requires that the results from the two studies be easily compared. However, this is not the case. For the field experiment, Kettler reports "bean yield" which appears to be bean *seed* yield (though this is never exactly specified); in the pot experiment he reports above-ground bean plant biomass. If a subsample of plants in the field experiment had been measured at 50 days to calculate a relationship between total plant biomass at 50 days and bean yield for each treatment, the results of the two experiments could have been more easily compared. To facilitate comparisons, I have presented the following results in terms

of P uptake and P input. Using data from Kettler's dissertation (1995), I have calculated percent P content for the beans harvested in the field plots, and using that value, I calculated total P harvested in bean seeds from the field plots. Because the pot experiment did not last as long as the field one, the results for the pot experiment are lower, as a group, than those of the field study. Nonetheless, by presenting the results as early-but-whole-plant-P-uptake (for the pot trials) and final -but-partial-plant-P-uptake (i.e., seed-only-P in the field trials), I am able to compare the two experiments at a similar scale in all the subsequent charts. Please note that treatments are referred to by the first letters of the tree species names or "T" for the *frijol tapado* treatment.

2.1. Assessing results from the field experiment

Figure 2.1 portrays the relationship between P input (in the form of slashed tree material along with slashed "weed" material) and P harvested from the field plots in the form of bean seeds.

The relationship between input and yield for the field results appears strikingly linear. Performing regression analysis on Kettler's means yields an $r^2 = 0.9024$, $P = 0.004$. Without Kettler's actual observations, though, this analysis does not do much more than corroborate the visual appearance of linearity. Kettler does present a correlation matrix in his dissertation (1995, p. 125), and in it the relationship between P input and total bean seed yield (not just P content

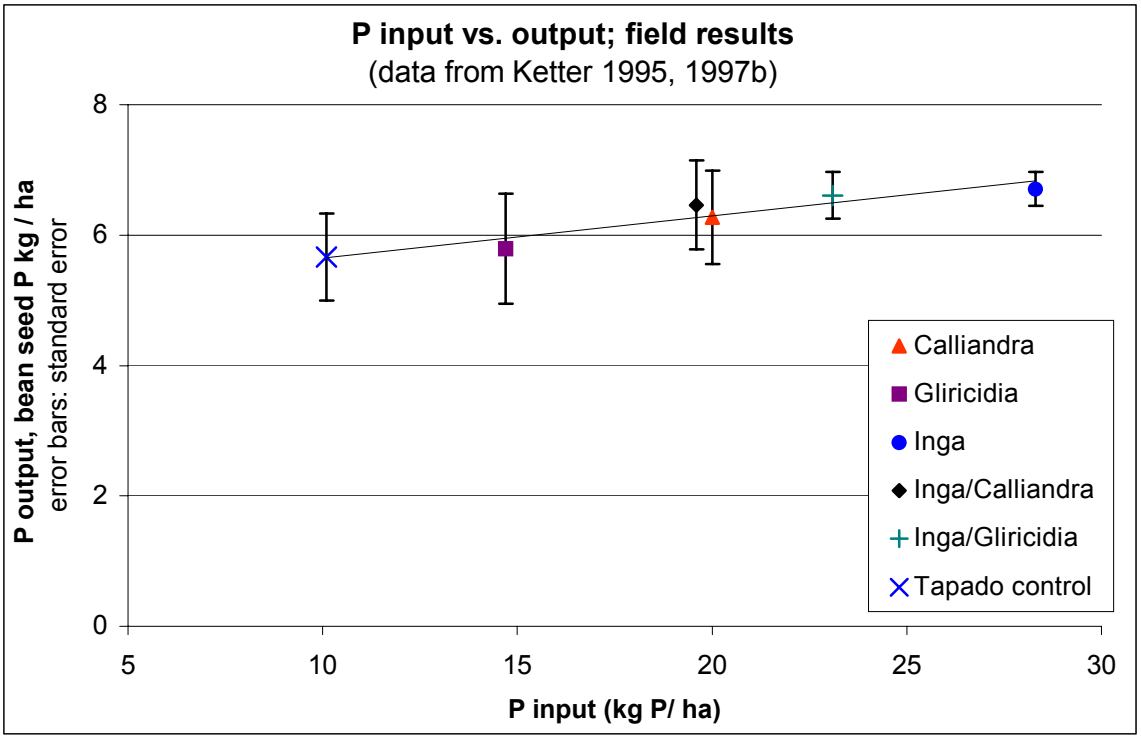


Figure 2.1 P content of surface-applied mulch from different agroforestry species and species mixes vs. bean seed P yield; field results, data from Kettler (1995, 1997b).

of the yield) is much less direct than the one I have presented here: the correlation coefficient he obtains is 0.3177. Furthermore, his mixed model analysis of variance showed no significant treatment effect ($P = 0.48$). It is clear that there must have been a great deal of variation within each treatment to produce this disparity in analyses. However, it is not clear that correlation (as opposed to regression) was the correct analysis for Kettler to perform given that the inputs were a controlled independent variable. A reanalysis of the original data might turn up some interesting relationships.

The possibility of such a linear relationship existing needs to be explored further. If it *does* exist, it would indicates that differences in mulch species (e.g., differences decomposition rate, lignin content, etc.) are largely irrelevant in the context of the *frijol tapado* system and possibly other slash/much systems. This would contrast sharply with the agroecological project of analyzing the relationship between plant litter quality, decomposition, and nutrient release so as to better tailor plant residue inputs for specific agronomic ends (Cadisch and Giller 1997). Most, if not all of that research has focussed on cropping systems where the seeds are sown into the soil and have most of their roots there. In the *frijol tapado* system, however, 85% of the bean roots are found in the mulch/litter interface (Rosemeyer and Barrantes 1992, in Kettler 1997a, p. 174). The possibility of such a difference existing between soil-sown and surface-sown systems points to an important research area: understanding nutrient dynamics in multiple layers of plant material as well as root survival and nutrient capture by roots embedded in those layers.

The relationship presented above is a special case of linear relationships: one that appears to have a slope of almost zero. If we take the regression of the treatment means at face value (i.e., ignoring for the moment the assumed large variability within the treatments), we obtain a regression slope of 0.065, or 15.76 when bean seed dry mass is used as the dependent variable. In other words, to obtain an increase in yield of 15.76 kg/ha, one must increase the mulch P application by 1 kg/ha, and

given an average tree mulch P concentration of 0.15%, this would require approximately 667 kg of added tree mulch. Compared to the tree mulch production figures that Kettler reports, this ranges from 5-10% of a given treatment's total production. I do not have the agroforestry nor economic data to determine the possibility or efficacy of trying to improve yields in this manner.

If we do *not* ignore the variability within the means, we are presented with a slope that does not differ from zero. There may be a subtle, but important distinction to be made here between there being (a) "no relationship" (dependent variable varies randomly across a wide range regardless of the independent variable) and (b) a specific relationship in which the dependent variable varies within a limited range across a range of independent values. The latter interpretation opens the door to many possible and testable hypotheses, including:

1) bean yields were not nutrient limited on the sites and at the times of this experiment (Kettler 1995, p. 115). This hypothesis can be further broken down into questions about which factors are limiting.
2) Or, to the contrary, the bean production under this system *is* nutrient limited:
    a) so severely nutrient limited that all the tested input values are below the requirements for showing improved yields (this seems unlikely);

b) or, more likely and more interesting: the crops are nutrient limited at particular crucial times during their growth, and none of the different mulch species releases nutrients in sufficient quantities at these times.

Kettler comments that the value of the tree treatments will only become evident after several years of harvesting from the same site.

In Figure 2.2, I have added the data points from Kettler's first bean harvest when the trees were still growing and the only mulch applied was

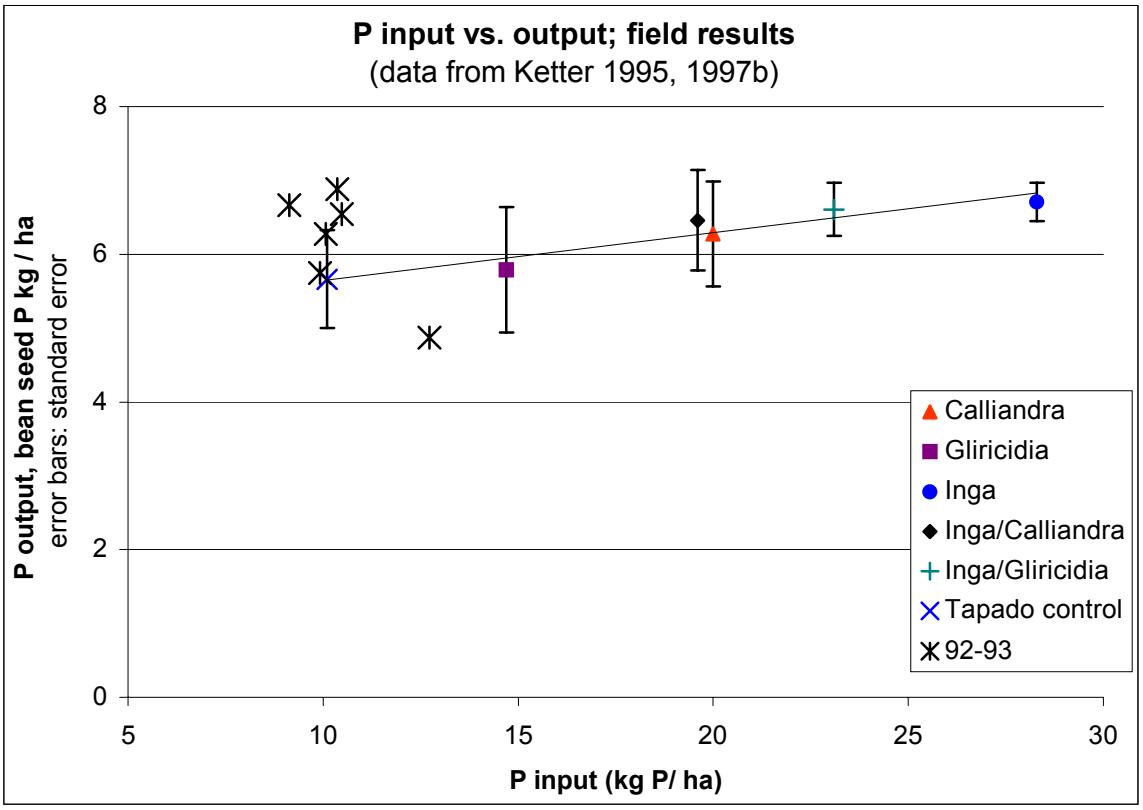


Figure 2.2: P content of surface-applied mulch from different agroforestry species and species mixes vs. bean seed P yield; field results, data from Kettler (1995, 1997b). including 1992-1993 results from tree plots without tree mulch (equivalent to *Tapado* Control)

from the volunteer secondary vegetation. The addition of these data appear to further corroborate the interpretation that bean yield varies across a limited range regardless of P input under the conditions tested.

2.2. Assessing results from the pot experiment

Figure 2.3 portrays the relationship between P input (in the form of slashed tree material) and P harvested from the pots in the form of above-ground bean-plant P.

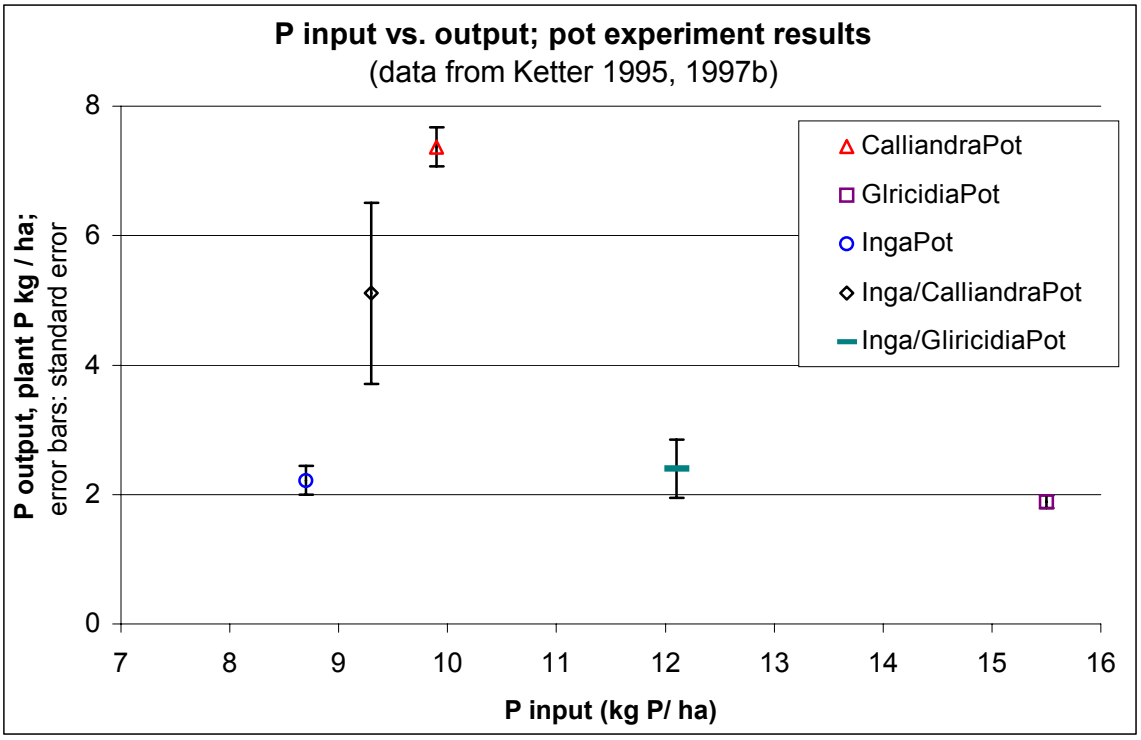


Figure 2.3: P content of surface-applied mulch from different agroforestry species and species mixes vs. bean plant P uptake; pot experiment results, data from Kettler (1995, 1997b).

At first glance, the pot-experiment results depicted in this figure show no clear pattern. However, closer inspection indicates that only one

of the treatments may be responsible for the deviation from a linear relationship between inputs and yield: *Calliandra*. The pure *Calliandra* treatment has the highest yield/input ratio of all the pot treatments (and all the field treatments, for that matter). Not surprisingly, the other treatment containing *Calliandra* (the mixed *Inga*/*Calliandra* treatment) has the second highest ratio in the pot experiment. It is not clear why the *Calliandra* treatment would behave so differently from the others. It has the lowest N content of all the treatments and one of the lower, if not the lowest, P content (Table 2.1). The *Gliricidia* treatment, however, with the highest N and P contents, does not show any signs of behaving differently from the other two non-*Calliandra* treatments (*Inga* and *Inga*/*Gliricidia*).

Table 2.1: Nutrient content of mulch material used in Kettler's pot experiment (Kettler 1997b), two different calculation methods using Kettler's data.

Treatment	nutrient input / dry mass input (Table 3)*		nutrient concentration (Table 2)*	
	%P	%N	%P	%N
Calliandra	0.13	2.38	0.14	3.27
Gliricidia	0.21	3.39	0.19	4.36
Inga	0.12	2.79	0.15	3.69
Inga/ *Calliandra*	0.12	2.59		
Inga/ *Gliricidia*	0.16	3.09		

*Note: I do not know why there is this discrepancy between the values I calculated from Kettler's data and those which Kettler reports (1997b). However, these differences do not alter the interpretation of the behavior of the *Calliandra* treatment.

Removing the obviously unique response of *Calliandra* leaves three data points which may in fact exhibit a linear relationship between input and P

uptake, though basing any conclusions about linearity on only three points is obviously unwarranted.

2.3. Comparing the results of the field and plot experiments

The following figure presents all the pot and field data together for the first time.

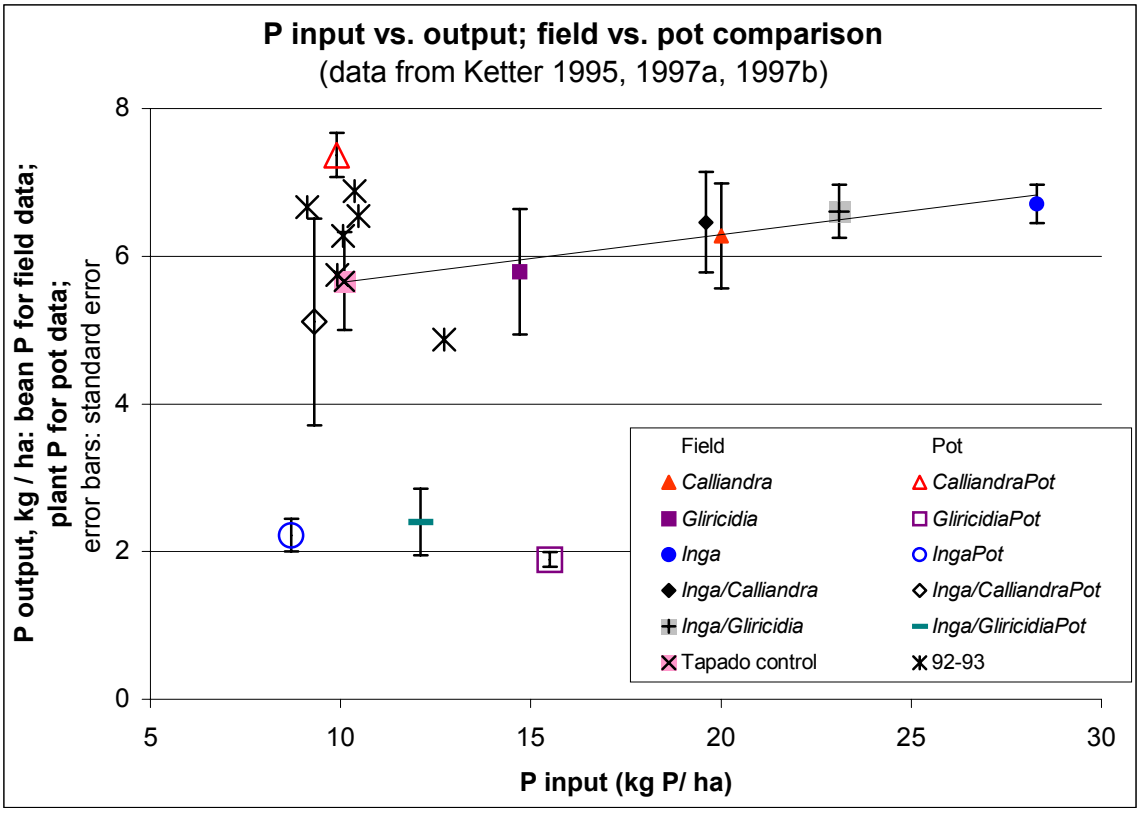


Figure 2.4:. P content of surface-applied mulch from different agroforestry species and species mixes vs. bean plant P uptake (pot results) and bean seed P conents (field results); field vs. pot comparison, data from Kettler (1995, 1997a, 1997b).

Although I have been able to place all these data on one graph using some simple data manipulations, the designs of Kettler's two experiments do not lend themselves to easy comparison, as I mentioned above, and it is

difficult to isolate the effects of this problem. Differences in treatment responses between the experiments may tell us more about the experiments themselves without teaching us anything new about the nature of the treatments being studied. Specifically, differences between the field and pot results could be largely the consequence of the following design decisions:

1) different levels of mulch were applied to the same treatments across the two experiments;
2) the mulch in the field plots contains mulch from the unplanted secondary vegetation "weeds" while this is completely absent, even as a control; and
3) the presence, in the field plots, of a litter layer of decomposed plant material from the previous year's weeds. There was no similar layer in the pot trials.

The first of these design decision, in particular, appears to have limited the usefulness of the pot study significantly. In the field experiment, the amount of mulch applied in a given treatment was dependent on the mulch production of the trees used in that treatment. In the pot experiment, the amount of mulch applied was equivalent in dry mass across all treatments. Given that the different mulch species have different nutrient contents, holding dry mass constant across the pot treatments necessitates that the different treatments are receiving different quantities of nutrients. There is nothing inherently wrong with this: the field plots also received

different quantities of nutrients. However, the different levels of nutrient inputs in the pot treatments are not related in any *systematic* way to the different levels in the field treatments. As a consequence, some of the pot treatments received more nutrients per area than same field treatments, while others received a third of their field counterparts. Table 2.2 documents this variation across treatments. (The reader will note that there are some discrepancies *within* the treatments, and I can only assume that these are due to the presence of weed material in the field values. This material, having different C:N:P ratios from the tree material would contribute differently to the denominator of each column.)

Table 2.2: Comparing nutrient and dry mass input between the pot and field experiments

Treatment	Pot / Field Input (all original values in kg / ha units)*		
	dry mass	P	N
Calliandra	57%	50%	57%
Gliricidia	69%	105%	137%
Inga	34%	31%	37%
Inga/*Calliandra*	54%	47%	55%
Inga/*Gliricidia*	44%	52%	51%

*Note: Field P and N input data are from Tables 4.9 and 4.10, respectively, in Kettler's dissertation (Kettler 1995). The other data are available in the published papers (Kettler 1997a, 1997b).

The consequences of this design decision can be seen clearly in Kettler's comparison of the field and pot results for *Inga* (Kettler 1997b, p. 1280): "The *Inga* treatments performed not nearly as well in the pot study as they did in the field." He goes on to propose that this observed difference is the consequence of *Inga*'s high lignin content inhibiting

decomposition in the disturbed microbial community of the soil container. He concludes: "The pot study demonstrates the slow rates of decomposition and subsequent nutrient element availability of single and mixed *Inga* treatments." I would argue, instead, that the pot study demonstrates that a pot treatment receiving approximately 30% of the nutrient input it received in the field will do worse than the other pot treatments which all received approximately 47%-137% of what they received in the field. The appropriate values for comparing these two experiments are nutrient-use-efficiency (nUE) values, i.e., how much output is produced per unit of input. Kettler provided these data for the field experiment in his dissertation (Kettler 1995, p. 127), but for some reason they do not show up in the subsequent publication (Kettler 1997a). I have reproduced them below along with my own calculations of nUE values for his pot experiment (Table 2.3).

Table 2.3: Comparing the P-use-efficiency (PUE) of Kettler's tree mulch treatments in the field vs. in pots; P output (plant or seed P) / P input (P total content of mulch); expressed as %s. The final column (Pot/Field) allows a comparison between the two experiments: values > 100% indicate that the treatment showed greater PUE in the pot than in the field; > 100% indicate lower PUE in the pot experiment. Note that P "output" in the field experiment was measured as P content of harvested beans after three months; whereas in the pot experiment, P output was measured in bean plant P content after 50 days.

Treatment	Pot	Field	Pot/Field
C	74	33	226
G	12	40	30
I	26	24	106
I/C	55	34	162
I/G	20	28	71
T		51	

Here we are able to see what I would argue is the *true* difference in *Inga* treatment performance between the field and plot experiments: *none*! Both exhibit approximately 25% use of the P applied. What needs explaining is not why *Inga* did so poorly but rather why *Calliandra* did so well.

The other two treatments he mentions in his comparison of the two experiments warrant some attention: *Gliricidia* and *Calliandra*. He attributes the different responses of the *Gliricidia* treatments (better in pots than field) to the relatively small amount of *Gliricidia* mulch produced by the trees for use in the field. There is no doubt that this is true given that the trees contributed approximately only 2% of the mulch dry mass in the field experiment, whereas they contributed 100% in the pot experiment. For this reason, there is no point in further analysis of the significance of the low PUE of the *Gliricidia* and *Inga*/*Gliricidia* treatments.

The issue of real interest is the response of the *Calliandra* treatment, or, more accurately, the difference between its response and *Inga*'s response. One possible explanation for the fact that *Inga* exhibits a similar PUE across the field and pot experiments while *Calliandra* does not is that the latter is more sensitive to microenvironmental conditions. Having worked with both *Calliandra* and *Inga* material, I suspect that this is the case. I have found that the relatively thick and large *Inga* leaves produce their own microenvironment which will be largely homogenous across a relatively rough landscape: the top layer of leaves generally

produces a barrier which appears to moderate temperature and moisture beneath it. The light, feathery leaves of *Calliandra*, on the other hand, do not really produce structured layers while decomposing on the soil surface. Rather, they dry out quickly (if weather permits) and accumulate in patches (leaving behind bare spots) as a result of wind and water movement. I am guessing that when *Calliandra* mulch is applied in the relatively protected environment of a soil container, it does not blow around leaving bare patches, and it decomposes (and releases nutrients) in a very different way that it does when it is applied in the field. Because no decomposition measurements were taken in either experiment, there is no way to test this hypothesis with the available data. Nonetheless, it warrants further study.

We may be able to gain greater insight into the *Inga* and *Calliandra* treatments by examining the behavior of the mixed-species treatment: *Inga*/*Calliandra*. In the pot experiment, the PUE for *Inga*/*Calliandra* is almost exactly halfway between the values for *Inga* and *Calliandra*. In the field experiment, though, it is approximately equivalent to the PUE for *Calliandra*.

PUE:

Pot: $I < I/C < C$

Field: $I << I/C \cong C$

The intermediate value in the pot experiment may be corroborating evidence for the "microenvironment" hypothesis: within a protected

container, *Calliandra* will contribute nutrients in direct proportion to its contribution to the mulch mass. The PUE behavior in the field experiment is intriguing but more difficult to interpret. Again, I suspect that microenvironmental conditions have an important role to play: Perhaps when *Calliandra* leaves are embedded in the protected moist matrix of *Inga* leaves, they are able to decompose (and release nutrients) in a more spatially and temporally consistent manner. In other words, the *Calliandra* in the mixed treatment may be behaving more like the *Calliandra* in the pot experiment than the pure *Calliandra* treatment in the field experiment. Again, though, there is no way to assess these hypotheses without decomposition data.

This struggle to interpret these results highlights a critical problem with using nUE values: What is their causal status? To what extent are they a cause or consequence of the results we are trying to understand? It seems conceptually sound to use nUE values as part of an explanation of other results. However, it is much more difficult to determine which results can be used to explain the nUEs.

2.4. Building on Kettler's Research

The problem of causal status is critical in Kettler's research and any other work seeking to explain how and why nutrients move where they do. Unfortunately, Kettler did not address this problem adequately, and as a result, the interpretive strength of the work is limited. Specifically, Kettler set out to explain crop-plant nutrient uptake results using

measures of soil nutrient availability (P captured by anion-exchange membranes and N extracted with KCl). However, by measuring nutrient availability in the same containers where the bean plants were growing, he undermined the explanatory strength of these measurements. Nutrient availability, measured in containers with growing plants, is the net result of additions from the decomposing mulch *and* withdrawals by (a) plant roots and (b) adsorption by the mineral soil (in the case of P). In other words, these values are not independent of plant uptake and therefore they cannot be used to explain it. Kettler established a soil-only control against which to compare the mulch availability results, but this control was not enough. He needed to have several additional controls as well:

1) mulch-but-no-plant: to available nutrient pools without plant uptake;
2) optimal-plant-growth: to have a maximum possible bean P uptake against which to compare the uptake by the plants under the mulch treatments; and
3) plant-with-no-mulch: to measure how much of the plants' nutrient uptake came from the soil, and how much from the mulch; (preferably this control would have some sort of fake mulch to control for soil temperature/moisture confounds).

With such additional controls, he could have successfully separated the inputs to and withdrawals from the available nutrients pool, and thereby

would have had a set of independent variables against which to compare plant uptake. Given the number and complexity of experiments Kettler was already managing at that point, adding more treatments to this one was undoubtedly impossible. Nonetheless, without them, his "study investigating the relationship between tree mulch decomposition and nutrient element availability" cannot, by design, tell us anything about that relationship.

My research has been, in large part, an attempt to build upon and extend Kettler's work by (a) teasing apart some of these causal tangles and (b) examining previously unmeasured but potentially important components of the nutrient cycle in the *frijol tapado* system. To deal with the causal tangles, I have had to explore new methods for measuring nutrient dynamics in the decomposition process. As for measuring previously unmeasured components, I wanted to make sure my work incorporated the layer(s) of already decomposed mulch from previous cropping cycles, suspecting, along with other *frijol tapado* researchers, that it may play an important role in increasing the likelihood of a plant root capturing P leached from the fresh mulch before it becomes irrevocably bound to the allophanic clays in the mineral soil.

CHAPTER 3

DECOMPOSING MULCH AND NUTRIENT AVAILABILITY: A CONCEPTUAL AND METHODOLOGICAL REASSESSMENT

Though the nutrient-use-efficiency (nUE) values I discussed in the previous chapter are the only useful values for comparing the treatments in Kettler's field and pot experiments, they suffer from a major problem: They conflate both (a) nutrient release from the mulch and (b) the plant's capacity to make use of those nutrients. As a consequence, a low nUE could be obtained in either of the following drastically different cases: (a) a mulch which releases its nutrients so slowly that the plant can barely grow or (b) a mulch which releases its nutrients so quickly that the crop plant never has a chance to absorb them. Because they obscure such differences, the nUEs do little to increase our understanding of the processes responsible for the observed results. While they allow us to compare crop response to the specific mulches tested in these experiments, they do not reveal the relationships between mulch *properties* and crop yields. Without this information, it is impossible to predict crop response to as-of-yet untested mulches.

3.1. Measuring nutrient release in litter decomposition experiments

As I mentioned above, the information provided by nUEs needs to be separated into its component parts. Plant nutrient uptake rates vary across levels of nutrient availability and therefore need to be determined in their own independent experiment in which plants are exposed to a defined range of levels. In my research, I have focussed on the other component: nutrient release from the mulch. This information is obtained by measuring changes in mass and nutrient content of a decomposing substance over time--what is typically referred to as a "litter decomposition" experiment. Measuring these changes requires some means of (a) exposing the plant material to its surroundings while also (b) isolating it from its surroundings to maintain its existence as a measurable entity over time. Every possible method for measuring decomposition involves a tradeoff between these inherently countervailing goals. Typically, this is accomplished by enclosing a known amount of plant material in a chemically-inert mesh bag. Wieder and Lang (Wieder and Lang 1982) attribute the origins of this practice to a variety of researchers (Falconer et al. 1933, Lunt 1933, Lunt 1935, Gustafson 1943, Bocock and Gilber 1957, Bocock et al. 1960) and mention various researchers who discuss its limitations and inevitable tradeoffs (Witkamp and Olson 1963, Wiegert and Evans 1964, Witkamp and Crossley 1966, Ewel 1976, St. John 1980).

Nutrient dynamics in decomposing litter have typically been studied by measuring changes in the litter's nutrient content over time. The term "nutrient release" is almost always used synonymously with and as shorthand for "change in litter nutrient content," e.g., "Nutrient release from decomposing leaf and branch litter in the Hubbard Brook Forest, New Hampshire" (Gosz et al. 1973). The logic is simple and intuitive: (nonvolatile) nutrients lost from the litter must be released into the soil; there is simply nowhere else for them to go. The logic became more complicated when researchers were faced with the unexpected observation of *increases* in nutrient content of decomposing litter (Gilbert and Bocock 1960, Gosz et al. 1973). Apparently, for a time, these observations were so unexpected that they were rejected for publication as obvious mistakes and artifacts of sloppy measurements (Crossley Jr. 1996, Haines 2002).

By 1981, this once-surprising phenomenon had been studied enough for Berg and Staaf to summarize the results of 14 selected studies reporting nitrogen accumulation in forest-floor and tundra litter (Berg and Staaf 1981). Litter material from 26 deciduous and coniferous species had been studied, some species studied by more than one researcher. Out of the 46 materials studied in total, Berg and Staaf documented 12 which released nitrogen, eight which showed no change, and 26 which accumulated nitrogen. Maximum accumulation ranged from 110% to 300% of original, with an average of 151%, median of 140%, and standard deviation of 46%. Berg and Staaf also identified a general three-phase

pattern to describe the nutrient dynamics of decomposing litter: (1) abiotic leaching, (2) net uptake, and (3) net release (Figure 3.1).

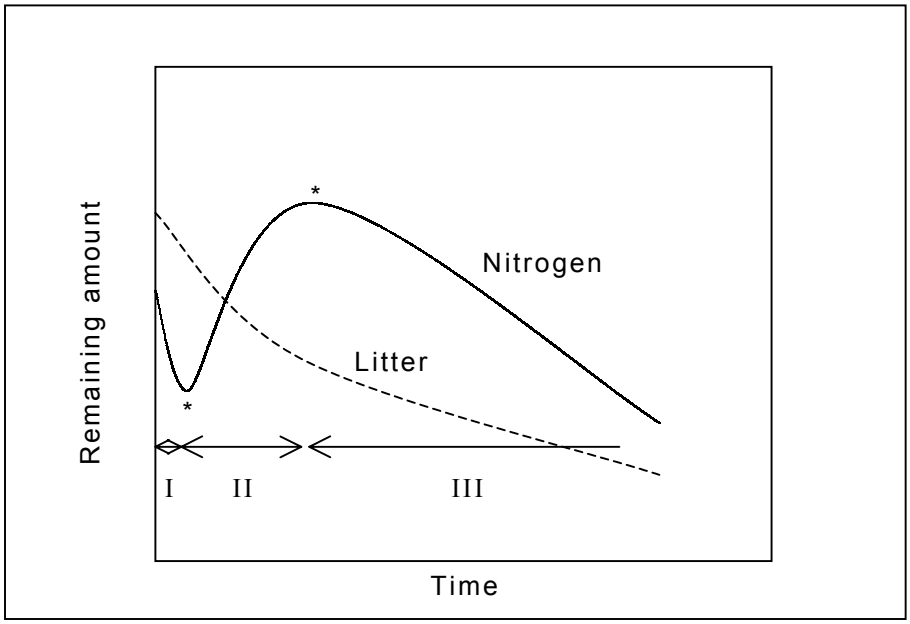


Figure 3.1: Illustration of the phases for leaching, accumulation and release of nitrogen from needle litter. I. Leaching; II. Accumulation; III. Release. (reproduced from Berg and Staaf 1981)

The accumulation of nutrients in decomposing litter is described as the result of two processes: inflow and retention (Gosz et al. 1973, Swift et al. 1979). Inflow refers to the movement of external nutrients into the litter by any of the following processes:

1) asymbiotic fixation (in the case of N),
2) absorption of atmospheric nitrogen,
3) throughfall,
4) wet and dry atmospheric deposition,
5) addition of insect frass,

6) immigration of decomposer biota with biomass nutrients from accumulated outside of the litter,
7) addition of external litter,
8) capillary flow from the soil solution,
9) fungal translocation, and
10) microbial immobilization (from the soil solution).

When Gilbert and Bocock first reported nitrogen accumulation in decomposing forest floor litter (Gilbert and Bocock 1960), they attempted a quantitative assessment of several of these sources, but having evidence against most of them and lacking it *for* any of them, they concluded: "These are all only tentative suggestions; the actual origin of the exogenous nitrogen and its mode of incorporation into the litter must await investigation" (p. 18). The exact same sentiment was expressed twenty two years later, by Melillo and Aber (Melillo and Aber 1982) and even more recently by Frey *et al.* (Frey et al. 2000).

"Retention" refers to the process by which nutrients in the litter, whether originally there or there as a result of the inflows discussed above, remain locked up in the litter. The single mechanism invoked to explain this is one of the mechanisms used to explain inflow: microbial immobilization. In this process, microbial decomposers, fueled by the new energy source of new substrate, grow and consume available nutrients. As long as the ratio of available energy to available nutrients in the substrate remains higher than that required by the growing decomposer community, the nutrients will be a limiting factor, and as such, they will be conserved within the community. While each individual decomposer is both

consuming and releasing these nutrients (immobilizing and mineralizing), the nutrients released by that individual will be consumed by another growing individual, such that, at the community level, there is no release. It is not difficult to see how, with access to nutrients in the soil solution, the growth of such a decomposer community would no longer be limited by the nutrients supplied in the litter. Any such immobilization by litter-residing decomposers of nutrients from the soil solution would represent an inflow of nutrients into the litter. Thus, it is clear that, with regards to microbial immobilization, the process of inflow is merely a special case and logical extension of the more fundamental process of retention.

Retention of nutrients in decomposing plant material as a result of microbial immobilization has been studied by agronomists since the late nineteenth century (see the "previous work" section in Doryland 1916 p. 320, and the "early concepts" section in Bartholomew 1965 p. 286). As discussed in the introduction of Aber and Melillo (1982) and Bartholomew (1965), the main concern of these researchers was determining the nitrogen "tie-up" capacity of different plant-material composts added to the soil: adding the wrong kind could result in complete nitrogen starvation of a crop planted into the amended soil. It was this research that Gosz *et al.* (1973) and Swift *et al.* (1979) tapped into to explain the otherwise anomalous-seeming results of increasing nutrient content in forest-floor plant litter. And it was their explanations that served as the conceptual framework for the subsequent research examining the

dynamics of this process (Berg and Staaf 1981, Aber and Melillo 1982, Staaf and Berg 1982). Although the focus on forest ecosystems was new, the concerns were not: How rapidly do the nutrients in decomposing litter become available for plant uptake? The research strategy taken in answering this question has been to look for chemical properties of the plant litter that need only be measured once but can be used to predict nutrient dynamics throughout the *entire* decomposition process. One of the earliest and most durable contenders for such decomposition-predictors has been the nutrient content, or energy:nutrient ratio of the material, typically measured as carbon:nitrogen or carbon:phosphorus, or C:N:P. This measure of litter "quality", i.e., its ability to serve as a source of nutrients for growing plants, is easily determined and continues to be used today even though serious problems have been observed with it (Berg and Ekbohm 1983). More sophisticated predictors have been proposed and studied which segregate the energy content of litter into more or less available pools, e.g., lignin and acid-insoluble-substances content (Aber and Melillo 1982, Berg and McClaugherty 1987, Berg and McClaugherty 1989), polyphenolics (Palm and Sanchez 1990), and combinations of all of the above (Tian et al. 1995).

3.2. Simultaneous inflows and outflows: an unacknowledged anomaly?

In 1988, Berg (1988) observed ^{15}N moving out of decomposing Scott's pine needles (*Pinus sylvestris*) at the same time that the absolute

amount of nitrogen in the litter was *increasing*. Similar results were reported by Blair *et al.* (1992) (Figure 3.2). These results demonstrate that N is *not* being retained in the litter at the same time that it is flowing in from external sources. Compare these results to, for example, the following statement of Swift *et al.* (1979):

> As decomposition proceeds the C:nutrient ratio will decline. C is lost continuously but the limiting nutrient will be conserved in an immobilised form. Conditions may soon be approached when the nutrient is no longer limiting and nett mineralisation becomes a possibility. *...The implication of this is that the release of inorganic forms of an element X will occur* ***only*** *when the C:X ratio of the resource drops to the level at which it is no longer limiting to the organisms decomposing it...*(p. 37, emphases mine).

I would argue that Berg's results and others like them actually *falsify* this proposed explanation/model of nutrient dynamics in decomposing litter. Furthermore, by falsifying this model, they uncover some potentially serious problems for litter decomposition research. If N is flowing out of litter even during the times when it should be being conserved by the microbial decomposers who are limited by its growth, then it is no longer safe to assume that one can determine changes in the pool of plant-available N by measuring changes in total N content of the litter. In other words, it is no longer safe to equate "nutrient release" with "litter nutrient content change." It certainly would not have been safe in the contexts that Berg (1988) and Blair *et al.* (1992) studied, and there is no way of knowing, without further study, where and when else it is not safe to

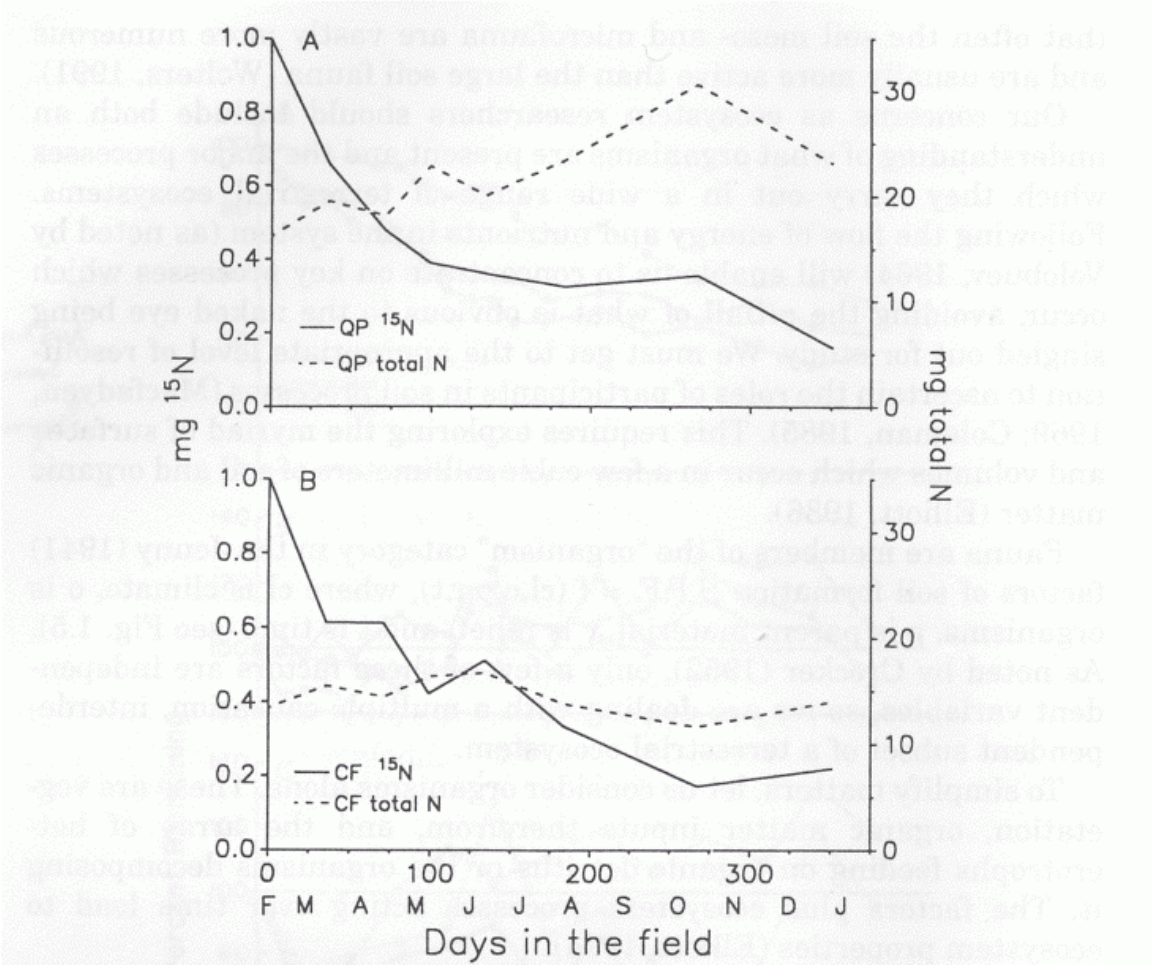


Figure 3.2: (Blair et al. 1992) <<insert standalone caption here>>

make this assumption. In other words, this assumption cannot be treated as safe in *any* context until proven otherwise. This seems to me to be a fairly radical development in the thinking on nutrient dynamics in litter decomposition, yet it does not seem to have been recognized as such in subsequent research. Of all the papers in the book *Driven by Nature: Plant Litter Quality and Decomposition* (Cadisch and Giller 1997), none cite Berg 1988, and the one that cites Blair *et al.* 1992 does not make mention of this consequence of their work (Wardle and Lavelle 1997). The

historical overview chapter mentions neither of them (Heal et al. 1997). Litter decomposition studies routinely equate nutrient release and changing nutrient content in the litter without any reference to the research that calls such a relationship into question (e.g., Seneviratne 2000, Regina 2001, Burgess et al. 2002, Tutua et al. 2002). Some researchers have in fact recognized the problems presented by Berg 1988 and Blair *et al.* 1992:

> Many decomposition experiments using litterbags have been performed in forests and rates of mass loss as well as dynamics of mineral element concentrations in litter are rather well known (Berg 1986). However, as external N may accumulate in litter, little is known about the rate and forms of litter N release and redistribution in the soil and the ecosystem. (Zeller et al. 2000, p. 551.)

Nonetheless, this recognition has not led to the reassessment of the litter decomposition models which do not, by design, allow for such simultaneous flows. In the following sections, I discuss *why* I think the importance of Berg's 1988 results have gone largely unexplored, and this discussion will lead to my attempt to modify the conceptual foundations of litter decomposition modeling to account for those results.

3.3. Why an "unacknowledged" problem? "Net" confusion

Many litter decomposition studies examining nutrient dynamics refer to change in litter nutrient content as "net" change (mineralization or immobilization). The use of this word "net" implies that the measurement accounts for simultaneous nutrient inflows to and outflows from the litter, making Berg's 1988 results seem obvious before they were

even obtained. However, it is clear that this notion of "net" is contradictory to Swift *et al.*'s (1979) model of nutrient conservation in decomposing litter in which "the release of inorganic forms of an element X will occur *only* when the C:X ratio of the resource drops to the level at which it is no longer limiting to the organisms decomposing it..." (p. 37, emphasis mine). It is not consistent with the repeated interchanging of the terms and concepts "net release", "release", and "increase in plant-available pools", e.g., "phase III" in Berg and Staaf's (1981) summary of nutrient dynamics in the decomposition process (Figure 3.1).

My assessment of the litter decomposition literature is that "net" has taken on two (at least) contradictory implicit definitions. These multiple definitions allow "net" to take on whichever conceptual role is demanded of it in any given moment (e.g., making Berg's 1988 results seem obvious in one context and paradigm-shifting in another).

In the agronomy and soil-microbiology literature, the "net" in net-mineralization and immobilization refers to the fact that:

> given:
>
> (a) microbial decomposers are continuously releasing and taking up nutrients
>
> and
>
> (b) microbial decomposers outcompete plant roots for nutrients on a surface-area to surface-area basis,
>
> then
>
> (c) plants never have access to the mineralization part of that flow. They have access only to the sum of mineralization and immobilization, i.e., the *net* outcome of those two flows.

In this context, "net" refers to the underlying microbial process but does *not* refer to anything relating to the larger ecosystem-context of nutrient availability for plants. In other contexts, though, "net" refers to the sum of litter nutrient inflows and outflows in which the outflows *are* plant-accessible. Both uses of the word "net" refer to sums of outflows and inflows, but only the latter has ecological significance beyond the scale of the microbial decomposers.

Berg's 1988 results are an example of the latter "net", which I will refer to as "net(plant-available)" for the rest of this chapter. The vast majority of litter decomposition research uses "net" in the former sense, which I will now call "net(microbial-turnover)". If the ^{15}N in Berg's pine needles had participated in net(microbial-turnover) flows, there would have been little or no change in the litter ^{15}N quantities over time: the ^{15}N released by one microbial decomposer would have been taken up by another. If that ^{15}N had leached out of the litter and entered into the soil solution, it must have entered a different pool of N than that which was the source of the N flowing *into* the litter. If they had entered the same pool, all or most of the ^{15}N flowing out would have flowed back into the litter. While it is certainly possible that the leached ^{15}N is being captured by *soil* microbiota, that cannot be *assumed* without further testing.

When Berg's results are assessed, they are typically seen as an another example of what everyone already knows: nutrient change in litter is a measurement of net change, and he is simply documenting this net

change. Yes, he *is* reporting a net change, but *not* the net change people think he is reporting.

For an example of net(microbial-turnover), I will refer to the CERES-N submodel <<need CERES-N ref here!>> as described in Quemada and Cabrera's (1997) work assessing the possibility of modifying this submodel for application to surface-applied crop residues. Mineralization and immobilization are calculated as two different flows, and thus it is feasible to talk of "net" mineralization or immobilization. However, closer analysis reveals that *all* the microbially mineralized N (which does not get turned into recalcitrant humified material) is immobilized in the very next computational step: "The N immobilized is the minimum of two values: soil inorganic N and the demand of N by the decomposition of fresh organic matter (FOM)" (Quemada and Cabrera 1997, p. 734). In other words, simultaneous inflows and outflows at the microbial temporal scale are *not* simultaneous inflows and outflows at the plant-root-uptake temporal scale. As far as the plant root is concerned, there are only one-way flows, either into *or* out of the litter, and the magnitude of those one-way flows is determined by microbial demand. So, yes, a "net" sum of two flows is being calculated, in one sense, but in a very important other sense, there is only one process or flow being measured: microbial demand. The two different calculations of mineralization and immobilization are calculations determining the impact of the microbial demand, but as far as the rest of the ecosystem is

concerned, those flows are inaccessible; they are part of a black box that has only one output. Given a specified level of microbial biomass (and its nutrient demand) and given a C:N ratio of the substrate they are decomposing, nutrients either (1) flow out of the litter into the soil solution (if the demand is exceeded), (2) flow into the litter from the soil (if the demand is not met), or (3) neither (if the demand is met exactly), but there is no way, by design, for any of these flows to happen simultaneously. The microbial box can have only one arrow coming out of it or into it at a time. In other words, there is simply no way to obtain Berg's 1988 ^{15}N results from the CERES-N submodel. Berg and CERES-N are both measuring the net results of two flows, but the flows are different, the meanings of "net" are different, and their ecological consequences are different.

The CERES-N submodel is a direct descendant of the agronomic and soil-microbiological decomposition research of the early twentieth century, and as such, it was never intended to describe nutrient dynamics in decomposing plant material on the soil surface. The Swift *et al.* (1979) conceptual model, and all the subsequent litter decomposition work based upon it, are *indirect* descendants of that same agronomic research. I say "indirect" because Swift *et al.*'s (1979) goals were different: they set out to move beyond the agronomic work to non-agricultural ecosystems; but, to meet those different goals, they adopted the agronomic microbial-nutrient-demand explanation for nutrient dynamics in decomposing litter.

In some ways, the work of Quemada and Cabrera (Quemada and Cabrera 1995, Quemada and Cabrera 1997, Quemada et al. 1997) is similar to that of Swift *et al.*'s (1979), in that they are all attempting to retrofit agronomic models for use in contexts not anticipated by the authors of those models.

The fact that Berg's 1988 results cannot be accounted for in either of these models highlights what I suspect is a critical flaw in the attempt to apply agronomic concepts to non-agronomic contexts. Specifically, there are two assumptions embedded within the Swift *et al.*'s (1979) conceptual model and the CERES-N submodel, that need some examining:

1. microbial decomposers have access to nutrients external to the litter, specifically nutrients in the soil solution, and
2. the growth of microbial decomposers is more limited by nutrient availability than by habitat availability.

I would like to suggest that these two assumptions are always, or almost always, met for decomposing plant material that has been incorporated into the soil. These assumptions were perfectly warranted in the agronomic context which spawned them. To a certain extent, they were *so* perfectly warranted, so clearly *obvious*, that they did not need stating. However, the authors of these assumptions never anticipated the uses to which their creations would be put. Because these assumptions remained unstated, they were imported silently into Swift *et al.*'s (1979) non-agronomic conceptual model, where, I would like to suggest, they have been wreaking quiet havoc ever since, undermining all efforts to use litter-quality measurements to consistently predict decomposition nutrient dynamics. Why? Because, I suspect, these assumptions are *not* always, and maybe even *rarely*, met for decomposing plant material *above* the soil

surface. In the next section, I will discuss what I suspect are the consequences of not meeting these assumptions and how litter decomposition models may need to be modified to account for contexts in which they are not met.

3.4. Litter quality: moving beyond litter chemistry?

I suspect that significant portions of above-ground decomposing plant material are:

1) *not* in contact with the soil solution (Figure 3.3.a), and
2) sufficiently hostile microenvironments for certain kinds of microbial biota that their growth is limited more by habitat availability than by nutrient supply (Figure 3.3.b vs. c).

If the litter solution is not continuous with the soil solution, then microbial decomposers in the litter cannot immobilize nutrients from the soil solution. In other words, no inflows can occur as a result of microbial immobilization. These decomposers may still be retaining and conserving all the available nutrients originating from the litter, tying them up in microbial biomass (Figure 3.3.d), but they cannot (1) *reduce* the pool of nutrients plant-available nutrients in the soil nor (2) retain any more nutrients than there were to begin with in their respective litter layer.

There may be, however, a serious limit to the quantity of nutrients that these decomposers can retain. If they were growing in the pores of a moist, aerated soil, they would be limited only by their food supply (Figure 3.3.e and f). But instead, they are growing in an environment

which experiences rapid fluctuations between extremes in temperature and moisture. These fluctuations may, in fact, hold their populations down well below what their food sources would allow them to achieve under better circumstances (Figure 3.3.g).

Combining (1) an inability to pull nutrients from the soil solution and (2) an inability to hold on to all the nutrients available in the litter results in the possibility of gross nutrient *release* (Figure 3.3.h and i) from litter which, according to the standard models, should be *accumulating* nutrients. If fungal translocation, faunal immigration, etc. are, at the same time, brining in nutrients from other soil and litter pools, these gross inflows and outflows will be occurring simultaneously outside of the microbial mineralization-immobilization turnover (MMIT) cycle. It is these processes, I suspect, which can explain the Berg 1988 results and which cannot be accounted for in any of the current models of litter decomposition.

As I mentioned earlier, it is entirely possible that when these nutrients released from the upper litter layers enter the soil solution (or its extension into the lower litter layers), they are being immediately immobilized by the *soil* microbial community (Figure 3.3.j and even k). If so, the journey outside the microbial turnover box did not get very far and is of little ecological significance. However, it cannot be *assumed* that this is in fact occurring. It is also entirely possible that plant roots, with their extant surface area, are better prepared to take advantage of sudden

and large nutrient inflows resulting from heavy rainfall than are the microbial communities which must grow new surface area before they can outcompete the plant roots as a whole. The fact that Zeller *et al.* (Zeller et al. 2000, Zeller et al. 2001) observed ^{15}N move from labeled beech litter to growing beech trees within six months while total N in the litter was increasing is indicative that possibilities such as my hypothetical example must at least be considered. Furthermore, there may be ecological contexts in which immobilization of litter nutrients by soil biota is avoided altogether: namely, those ecosystems (including *agro*ecosystems) in which plant roots are found in the litter layers or on the soil surface (Figure 3.3.l). These may include tropical rainforests with surface root mats <<Jordan refs here>>, forest canopies with their plants growing in non-mineral "soils" <<Nadkarni refs here>>, and, yes, *frijol tapado* with its bean roots in the decomposing mulch.

Are there variables we can measure in order to predict these non-microbially-mediated outflows from decomposing surface litter? Yes, I think so, but I do not think that they are going to be the standard measurements found in the "litter quality" literature. In fact, I suspect that they will not even be the same *type* of measurements. Specifically, I would argue that it is the *structure* of the plant litter (Figure 3.3.m) , rather than its *chemistry* (Figure 3.3.n) which determine:

1) where the boundary (Figure 3.3.o) lies between the two ecologically-distinct litter layers: (a) the one in contact with the

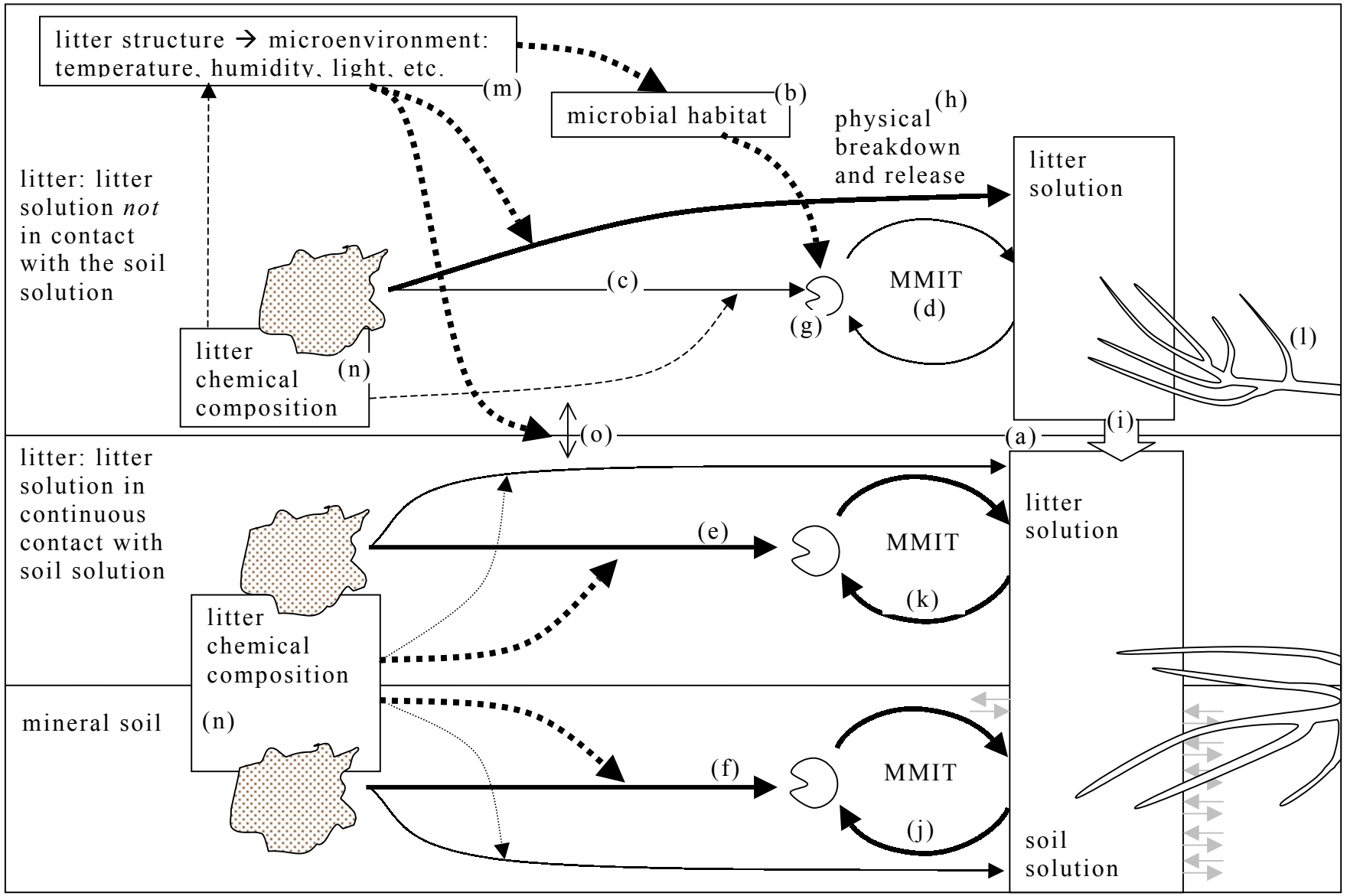
soil solution and (b) the one *not* in contact with it; i.e., how much of the litter needs to be characterized according to its structure and how much according to the standard chemical properties; and

2) the extent to which microbial growth is limited by habitat rather than nutrient supply (Figure 3.3.b).

Of course, structure is itself determined by chemistry, but I would argue that the chemistry→structure→microbial habitat explanatory chain is much more complicated than the chemistry→microbial digestibility one. In other words, I think it will be much more feasible to come up with structure determinants of habitat than chemical ones. Once the structural determinants have been established, research can focus on finding the chemical determinants for them. Therefore, I am advocating a move away from litter chemistry as the determinant of nutrient dynamics in plant materials decomposing on the soil surface. Models based on litter chemistry and microbial digestibility will still apply, of course, but only to soil-incorporated or soil-solution-contacting materials. It seems likely that studies of plant materials decomposing on the surface will have to work with both models, depending on the structure of the material. My suspicion is that many of the explanatory difficulties encountered in litter decomposition research have been the consequence of litter chemistry having opposing effects on nutrient release in the different litter layers: In the lower "chemistry" layer, high carbon content usually results in

nutrient immobilization and reduced availability for plants. In the upper "structure" layer, it is entirely possible that this high carbon content is responsible for creating a hostile environment for microbes and promoting physical breakdown and thereby *increasing* nutrient release and availability for plants. In this situation, trying to come up with a single "carbon influence" coefficient in a regression equation predicting nutrient release will be an exercise in frustration. In this vein, I strongly advocate that future research in litter decomposition clearly identify itself as focussing on surface or soil-incorporated litter and that future reviews and meta-studies not mix data from these two groups of studies (as do, for example, Aber and Melillo 1982, Seneviratne 2000).

Figure 3.3: Comparing nutrient dynamics during decomposition in upper litter layers vs. lower litter layers or in the soil. Solid arrows represent nutrient flows. Dotted arrows represent flows of causality. Heavier arrows are intended to represent larger flows than those represented by smaller arrows. Thus, the "physical breakdown and release" nutrient flow (h) in the upper litter layer is intended to be a larger flow than microbial consumption (c). Similarly, the influence of microbial habitat (b) on the size of the microbial population in the upper litter layer (g) is intended to be larger than the influence of the litter chemistry (n). In the lower litter layer and the soil layer, litter chemistry (n) is intended to be the main influence on microbial nutrient consumption and growth (e and f).


litter structure → microenvironment:
temperature, humidity, light, etc.
(m)
microbial habitat
(b)
physical
breakdown
and release
(h)
litter
solution
litter: litter
solution *not*
in contact
with the soil
solution
(c)
MMIT
(d)
(g)
(l)
litter
chemical
composition
(n)
(o)
(a)
(i)
litter: litter
solution in
continuous
contact with
soil solution
(e)
MMIT
(k)
litter
solution
litter
chemical
composition
(n)
mineral soil
(f)
MMIT
(j)
soil
solution

CHAPTER 4

DESIGNING AND TESTING A CLOSED-SYSTEM LITTERBASKET

4.1. Introduction

This study began its life as an attempt to answer a conceptually simple question in a very specific context: which mulches serve as the best source of nutrients for the bean crops planted in them by small-scale farmers in parts of southern Costa Rica. However, the more I considered the potential significance of Berg's 1988 paper, the more complicated answering the question became. Berg broke the assumed direct link between net change in surface residue nutrient content and changes in plant-available nutrient pools, and as a result, the standard approaches to measuring litter decomposition became less useful. So, instead of comparing nutrient dynamics of different mulches, as I had originally intended, I ended up having to ask *how* to compare nutrient dynamics of different mulches: What is the fate of nutrients originating in plant residue as it decomposes? What proportion of these nutrients become available for uptake by other plants (and/or other organisms)? This is a fundamental question both for ecology in general and agroecology in particular. In most circumstances, the standard approaches to that

question will suffice, but in a few, like *frijol tapado*, it will not. What are those few circumstances? How can we identify them? How will we know when the standard model is appropriate and when it is not? The research I am presenting here is a small attempt at starting the process of answering these questions.

From the outset of designing my research, I considered several criteria and constraints to be critical:

1) I wanted to work with fresh, not dried and ground, plant materials.
2) Similarly, I wanted to study these materials under close-to-field conditions rather than laboratory microcosm conditions.
3) I wanted to study the dynamics of phosphorus rather than nitrogen. There were several reasons for this:
    a) P is considered the limiting nutrient in the *frijol tapado* system, and in much of Central American agriculture in general.
    b) N is unlikely to be limiting in a system with a nitrogen-fixer as its crop and nitrogen-fixers providing mulch. In fact, N dynamics in *frijol tapado* may be limited by low P available for N-fixation.
    c) Providing P to crops has been identified to be a difficult problem for agroforestry systems: "A large number of screening and alley cropping trials in different climate-soil environments indicate that the prunings of several tree species contain sufficient nutrients to meet crop demand, *with the notable exception of phosphorus*" (Palm 1995, p. 105, emphasis mine).

Focussing on P, has its drawbacks, of course, and one of them is that the available traceable isotope, ^{32}P, has a short half-life that limits its usefulness in multi-week/month studies.

4) The researchers studying *frijol tapado* have long informally hypothesized that the litter layer under the mulch might be playing an important role in increasing the likelihood of a plant being able to grab P leached from the mulch before it comes into contact with the P-fixing mineral soil. This hypothesis was formally stated in Kenneth Schlather's dissertation (Schlather 1998, pp. 16-17).
5) Finally, I wanted to make sure that whatever methods I proposed would be usable in low-tech, low-capital contexts: other students or researchers working on small-scale agroforestry in remote and/or rural locations around the world.

4.2. General logic of and rationale behind the design of the closed-system litterbasket

The reduced utility of the net change values forces us to find ways to isolate or estimate gross flows. The obvious best means for doing so is using traceable isotopes (stable or radio), but for a variety of reasons (discussed above), this was not an option for me. My challenge was to see if I could find some way to do this estimating, or at least place some *bounds* on the gross outflow from the mulch, without having to resort to isotopes. I determined that doing so required me to design a litter

decomposition chamber that was a closed system and had at least one major one-way-only flow. To accomplish this, I started with the concept of the litterbasket (Blair et al. 1991) and combined it with a lysimeter. This modified litterbasket/lysimeter had two layers of plant material: fresh ("mulch") and old ("litter"), the former resting on top of the latter but separated from it by nylon mesh windowscreening material (Figure 4.1). These two layers rest on top of yet another mesh screen, suspended over a plastic bag to capture the rainwater leachates that flowed out the bottom of the plant material. Because of the high-P-adsorption capacity of the soils (Jin et al. 2000) in the region where I was working, I had to exclude soil from the system or it would have acted as an almost infinite sink for P, relative to the mulch and litter P flows. Having eliminated soil from my system, I had to provide some other source of decomposer biota for the decomposition of the mulch layer. This was another important reason for adding a second layer to my system, a layer of already decomposed plant material removed from just above the soil surface (referred to as "litter" subsequently).

Before this modified litterbasket can be used in full-scale trials testing multiple varieties of mulches as candidates for fallow-improving agroforestry species, it needs to be tested for the following:

1) comparability with existing standard methods (litterbags), and
2) capacity to provide the kinds of data desired.

Even with a litter layer, the absence of soil in my litterbaskets threatens to remove them so far from the reality of field conditions that the data would be useless. Therefore, it was necessary to compare the decomposition dynamics in my chambers to those observed in the typically-used litterbags placed directly into the litter/mulch layers resting on the soil surface. In anticipation of the possible extreme differences between the closed-system litterbaskets and the litterbags, I decided to add a third and intermediate type of decomposition environment to the experiment. These latter chambers had the same two layers of mulch and litter as the other, leachate-capturing, closed-system containers. However, they were placed directly on the soil surface (as opposed to being suspend above plastic bags), and therefore, were not closed systems. To summarize, the three methods compared were:

1) traditional litterbags, abbreviated as "LB" frequently throughout the rest of this document;
2) two-layer, closed-system, leachate-capturing chambers, referred to as the "Leachate Collector" treatment, or "LC", and
3) two layer chambers placed on soil surface, referred to as the "Soil Surface" treatment or simply "SS".

Comparing the decomposition dynamics of the traditional litterbags with those of the Leachate Collectors will show whether or not the conditions within the leachate collectors are sufficiently representative of a more-normal decomposition environment. Comparing them both to the

Soil Surface chambers will elucidate which of the following two sources of artificiality has a greater impact: (1) absence of soil or (2) being in a plastic tub (barriers to moisture and heat flow, lateral insolation, etc.).

4.3. The treatments

Having designed these closed-system, leachate-collecting, two-layer decomposition chambers, I wanted to use them to ask the questions outlined above. First, though, I needed to determine whether the chambers worked at all: Are the decomposition processes in these chambers at *all* like those which occur in litter bags? Do inflows occur in both the litter bags and my chambers? The most obvious comparison at this stage is to test chambers vs. litter bags. The two most important factors influencing decomposition in this comparison are

(1) presence of soil

(2) presence of plastic tub with a fine mesh barrier on the bottom

Table 4.1. Distribution of principle factors influencing decomposition when comparing litterbags with my two-level, leachate-collecting, closed-system decomposition chamber

treatment	presence of soil	in a plastic tub
LitterBag	Yes	No
LeachateCollector	No	Yes

At this point I realized that I might be able to better tease apart the influence of these different factors by adding a third treatment: I could place the upper two levels of the decomposition chamber on top of the

soil surface. This was the birth of the SoilSurface treatment. Now the distribution of factors has an intermediate position as well as the extremes already proposed (see Table 4.2).

Table 4.2. Distribution of decomposition-influencing factors with the addition of a third, intermediate treatment: the SoilSurface treatment.

treatment	presence of soil	in a plastic tub
LitterBag	Yes	No
SoilSurface	Yes	Yes
LeachateCollector	No	Yes

At some point I realized that, with this experimental design, there would be no way to know how much leachate P had been contributed by the mulch and how much by the litter. To resolve this problem I included a quasi-treatment which I called "blanks". As the name indicates, I don't consider this to be a real treatment. Rather these "blanks" were intended to provide an averaged correction factor to subtract from the leachate-P values generated in the free-standing leachate-collector chambers. As such, I did not make a full treatment's-worth of these chambers. In fact I didn't even have enough material to make a half-treatment's-worth. It is fortunate that I thought to include these chambers; it is unfortunate that I didn't realize how important they would turn out to be. Only once I got all the conceptual algebra worked out did it become clear that the blanks would play a crucial role in determining whether or not there had been any P inflows to the mulch.

What I call a "blank" was made by using a false, inert mulch in the top layer of the chamber (chopped up plastic grain-sack material), and filling the bottom layer with the same litter that had been used in all the other LeachateCollector and SoilSurface chambers (Figure 4.2).

One other pseudo-treatment was included: "rain blanks". These were nothing more than empty versions of the LeachateCollector treatment. The plastic bags at the bottom of these "rain blanks" would capture any atmospheric P-inputs as well as any contamination originating from the chambers themselves.

See Table 4.3 for a summary of the treatments and "correction factors" with their respective replicates and the labels commonly used in my diagrams and elsewhere.

Table 4.3. Treatments and "correction factors"

label	description
treatments (n=10)	
LB	Litterbags
SS	Soil surface chambers
LC	Leachate collectors
pseudo-treatment correction factors (n=4)	
BL	"Blanks" (fake plastic mulch in the top layer, same decomposed litter in the bottom as in all the other treatments)
RB	Rain Blanks

Table 4.4. Summary of plant material and isolating container used in the different treatments and "correction factors"

	LitterBags	Soil Surface	Leachate Collector	Blank	Rain Blank
mulch	mulch in mesh bag	mulch in top level of plastic chamber	mulch in top level of plastic chamber	chopped up plastic grain sack in top level of plastic chamber	nothing in top level of plastic chamber
litter	not isolated and therefore not measurable	litter in lower level of plastic chamber	litter in lower level of plastic chamber	litter in lower level of plastic chamber	nothing in lower level of plastic chamber

The isolating containers were filled from homogenized stocks of mulch and litter. Subsamples were taken from the homogenized stocks prior to the filling of each container. These subsamples were used in determining the initial state of the variables measured.

The containers were placed in the field on 14 August 1999. They were removed on 5 September 1999. Because of a backlog of sample processing from the initial batch, I could not begin processing subsamples from these containers right away. In the APPENDIX , I document my analysis of whether the containers were affected by a "time of processing" effect.

4.4. Measured variables

For each layer of each replicate, I measured the following variables at the beginning and the end of the decomposition period:

1) dry mass (DM)

2) total P (TP)

3) bicarbonate-extracted P (BEP)

4) chloroform-fumigated BEP (CFBEP)

By subtracting BEP from CFBEP, one should obtain a measure of microbial P. This method has been used for soil microbial P (McLaughlin et al. 1986), but at the time of designing this experiment (Coleman 1997, Crossley Jr. 1997, personal communication), no one had used this method to determine microbial P living in and on decomposing plant material. Of the 36 papers that have cited (McLaughlin et al. 1986), only one appears to have reported using the method this way (Qiu et al. 2002).

The leachate collected from the (1) modified litterbaskets, (2) false mulch blanks, and (3) rain blanks will be analyzed for total P content. If necessary, leachate P can also be calculated from the other variables measured because the leachate collectors are closed systems.

4.1) P leached = mulch P lost + litter P lost

4.5. The questions

4.5.1. Decomposition in chambers vs. litter bags

The purpose of the first component of this study is simply to assess the realism and comparability of the decomposition results I obtain from my leachate collectors. The limitations, artifacts, and inherent tradeoffs of the litterbag method have been well discussed (e.g., Witkamp and

Olson 1963, Wiegert and Evans 1964, Witkamp and Crossley 1966, Ewel 1976, St. John 1980). Do my containers introduce too many more artifacts, or can they safely be used instead of litterbags?

To answer this question, I will simply compare the percent loss of a given variable in the fresh plant material ("mulch") in the leachate collectors vs. the litterbags over the course of the experiment: dry mass, total P, BEP, CFBEP. Given the somewhat experimental nature of the two bicarbonate-extraction values, the results from those pools will be given less weight than the other two, commonly measured, variables.

If a difference is found, I will compare the same variable of the mulch from the leachate collectors, litterbags, *and* soil surface containers in order to establish whether the difference is due more to (1) lack or soil or (2) the artificial microenvironment of the plastic container.

4.5.2. Estimating P flows during mulch decomposition

Once the leachate collectors have been tested for reasonable comparability with litterbags, I can turn to the more interesting question of whether or not my leachate collectors have helped us learn more about P dynamics during decomposition than we could have learned with the only the litterbags (Figure 4.3 and Figure 4.4).

4.5.2.1. Net accumulations

Do any of the treatments show net accumulations of P in the mulch? Do *some* of them show net accumulations? If so, what can we learn from

which ones do and which do not? Presumably, different net accumulations between treatments will be indicative of different sources and/or pathways of P entry, e.g., lateral transport, originating from the soil, originating from the litter. This is simply a small part of the larger project of determining the general conditions under which net accumulations occur. If they occur in my treatments, then we have proven without a doubt that they *can* occur, though there is no way to predict when they will occur again, without first doing further study into their mechanisms. However, if they don't occur, I will be in the sad position of not having proven or falsified anything generalizable. At least not about *net* accumulations.

4.5.2.2. Gross flows between the mulch and litter

All is not lost if I do not observe any net accumulations, though. Perhaps the most important reason for having the Leachate-Collectors is for this very situation: for being able to detect gross flows in to and out of the mulch when, normally, the lack of any net accumulation would lead one to believe that there are flows only out of the mulch, not in to it.

Intuitively I had suspected that I would be able to estimate or at least bound the range of possible values for gross P flows into and out of the mulch by using a closed-system litterbasket. However, I did not get all the conceptual algebra worked out to determine whether it could really be done until recently.

Net fresh-to-old transfer is calculated as follows: First, we assume that the change in the mulch (m1) P over the course of the experiment

(m1PChange) can be divided into two values: the amount of change we can measure directly (m1PLeachedKnown) and that which we are trying to estimate (m1PLeachedUnknown) (Figure 4.3).

4.2) m1PChange = m1PLeachedKnown + m1PLeachedUnknown

Although I am calling it "m1P*Leached*Unknown", this value represents a net transfer, the sum of leaching to and inflows from the litter layer. I have abbreviated this value as *q* because its value is is what is in *q*uestion here:

4.3) m1PLeachedUnknown = net mulch-to-litter transfer = GrossInflowFromLitter -GrossOutflowToLitter = *q*

This value is conceptually identical to that of net nutrient change in a litterbag: the only way to definitively isolate the inflow from the outflow is through the use of traceable isotopes. However, I am hoping that it will be possible to *bound* the range of values *q* can take as a result of its controlled and closed context. Rearranging equation 4.3, we get:

4.4) *q* = m1PChange - m1PLeachedKnown

The individual parts (and subparts) of this equation are worked out below:

4.5) m1PChange = m1iP - m1fP

where "i" refers to "initial" or the value of the variable at the start of the experiment, and "f" refers to the value at the final sampling time. So,

simply put, "m1PChange" is calculated by subtracting the final P from the initial P in the fresh plant material layer. The next component of determining *q*, m1PLeachedKnown, is calculated as follows:

4.6) m1PLeachedKnown = m1m2PLeached - m2BLPLeached

where "m1m2PLeached" refers to the amount of P leached from both the fresh and old plant material layers during the course of the experiment. This was discussed in more detail above, and specifically in equation 4.1. Here I present it in a slightly different manner, using the notation "m1m2" to indicate that the value is the combination of the "m1" value (fresh material) and "m2" value (old material):

4.7) m1m2PLeached = m1m2iP - m1m2fP

where:

4.8) m1m2t0P = m1iP + m2iP
4.9) m1m2t1P = m1fP + m2fP

The second component of "m1PLeached" is "m2BLPLeached":

4.10) m2BLPLeached = m2iBLP - m2fBLP

Where "BL" refers to the values observed in the "Blank" containers. As explained previously, the "Blank" containers have only old plant material in them. Instead of fresh plant material, they have a layer of chopped plastic material resembling the fresh material, in size and packing properties, used in the experimental treatments. Therefore, knowing how

much P leaches out of the BL containers allows us to estimate the quantity of P leaching out of the old material ("m2") layer in the Leachate-Collector containers. In other words, for equation 4.6 to be true, we have to make the following assumption:

4.11) m2PLeached in Leachate-Collectors ≅
m2PLeached in the false mulch Blanks

A more accurate version of this equation would be:

4.12) m2LCPLeached = m2BLPLeached +
EffectOfM1LeachatesOnM2Decomp +
EffectOfPlasticM1OnM2Decomp

These correction factors need to be determined in subsequent studies using similar methods. For now, we have to be satisfied with equation 4.11.

Now we know that we can calculate *q*, but does it tell us anything we cannot already find out by using litterbags? From equation 4.4, we can conclude something potentially interesting: Given that *q* is the difference between m1PChange and m1PLeachedKnown, if there *is* a difference between them, we know for a *fact* that there have been gross outflows and/or inflows which could *never* have been observed using the standard litterbag method.

1) If $q > 0$, then m1PChange > m1PLeachedKnown. In other words, more P left the mulch layer than can be accounted for in the blank-corrected leachate. This result is not directly relevant to the

primary question (isolating inflows and outflows) because we have not learned anything about the mulch layer we couldn't have learned from a litterbag. However, a positive q value sheds some light on the capacity of the litter layer to "store" P, presumably by slowing its movement into the P-fixing mineral soil and thereby prolonging the time it is available to plant roots. Or course, though, without multiple sampling times, there is no way to know how long that P would remain in the litter layer. Presumably estimates could be made based on water-holding capacity and flow-through rates.

2) If $q < 0$, then m1PChange < m1PLeachedKnown, indicating a net movement of P from the litter layer up into the mulch layer, i.e., "inflow" during decomposition. In a traditional litter-bag decomposition study, the only time that such an inflow can be demonstrated to have occurred is when the total P (or any given nutrient) in the plant material becomes greater than the initial quantity. Even in that circumstance, it is difficult to extract much useful information from the data: there has been a net inflow, but we have little, if any, ability to estimate the gross outflow (other than educated guessing). Presumably it is this hidden outflow which is the source of many of the nutrients taken up by plant roots in the mulch, litter, and soil. In order to better predict plant growth in agricultural and non-agricultural ecosystems with large quantities of litter turnover, we need to be able to better estimate the quantity

of nutrients available to those plants, which, in turn, I suspect, requires us to do a better job of isolating and estimating gross nutrient outflow from freshly deposited plant material.

4.5.3. The role of the litter layer in the P dynamics of decomposing mulch

Several calculations can be made as part of the effort to determine whether the litter layer retains inorganic P leached from the mulch layer. The first, mentioned above, is to calculate q, and if it is positive, we know that we have added at least that much P to the litter layer. Of course, some of this positive q will probably be in the form of particulate P--pieces of mulch that have become small enough to fall through the mesh holes. To correct for this, it should be possible to compare the dry mass changes in litter below mulch vs. litter below false plastic mulch.

4.5.4. The role of microbial decomposers in the P dynamics of decomposing mulch

Given that microbial P had not been measured in decomposing plant material using the chloroform-fumigation, bicarbonate-extraction method, I did not know what to expect from this part of the experiment. I suspect possible confounds from the effect of chloroform on plant cell remnants, and the magnitude of these confounds will probably be related to the amount of exposed cross-sectional leaf area--a property which will

undoubtedly change during the process of decomposition. Therefore, I expect this component of the experiment to do little more than generate useful data about the variability of these kinds of measurements, and whether the values obtained seem reasonable relative to other values. With those data, it should be possible to further refine the method and calculate appropriate sample sizes for future work.

If the microbial P values appear to be useful, I will check them against the estimated gross flows between the mulch and litter layers. If there is a positive q, is there any correlation between q and the change in size of the microbial P in the litter?

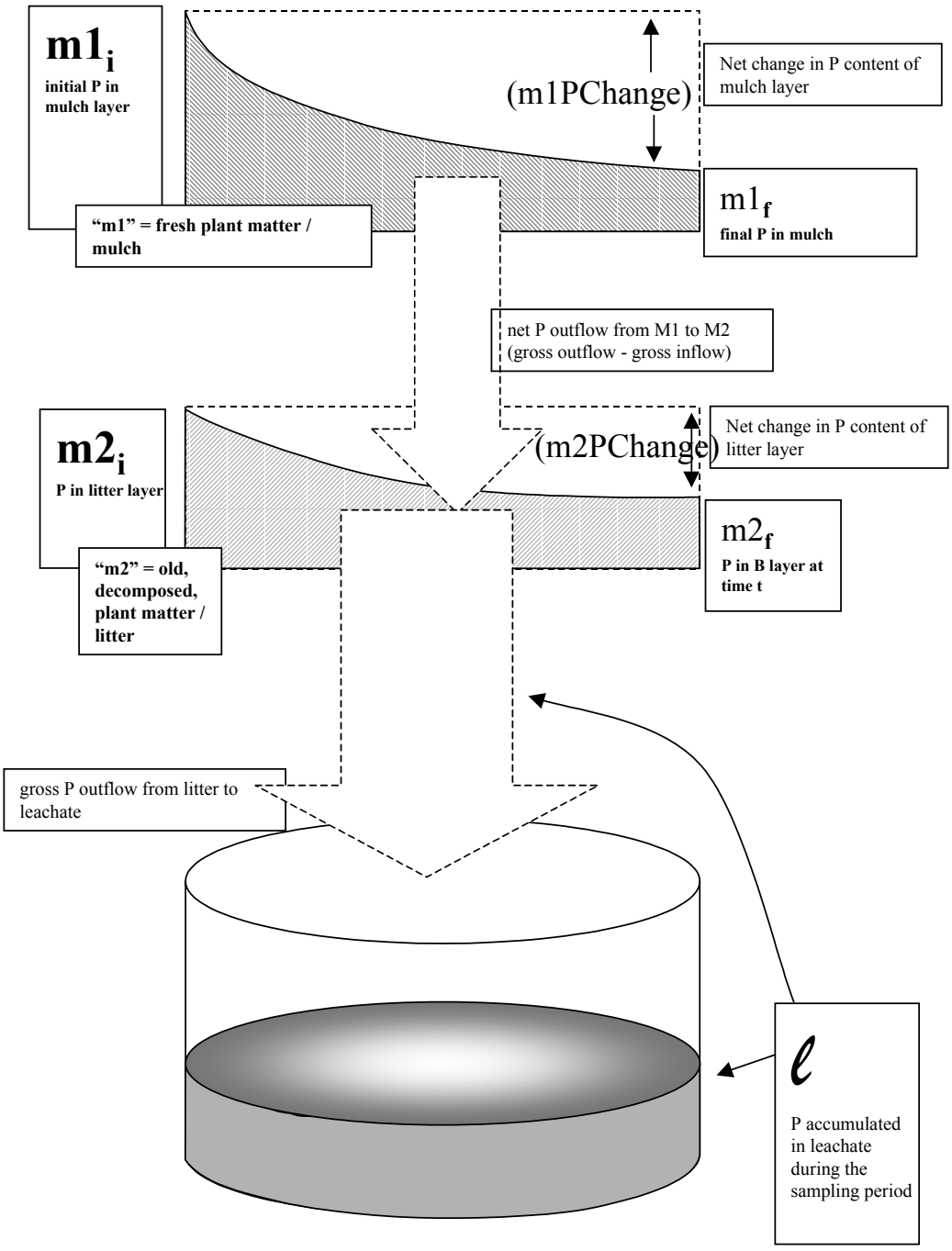


Figure 4.1: P dynamics in two-layer, closed-system decomposition chambers: measured variables in a sample chamber

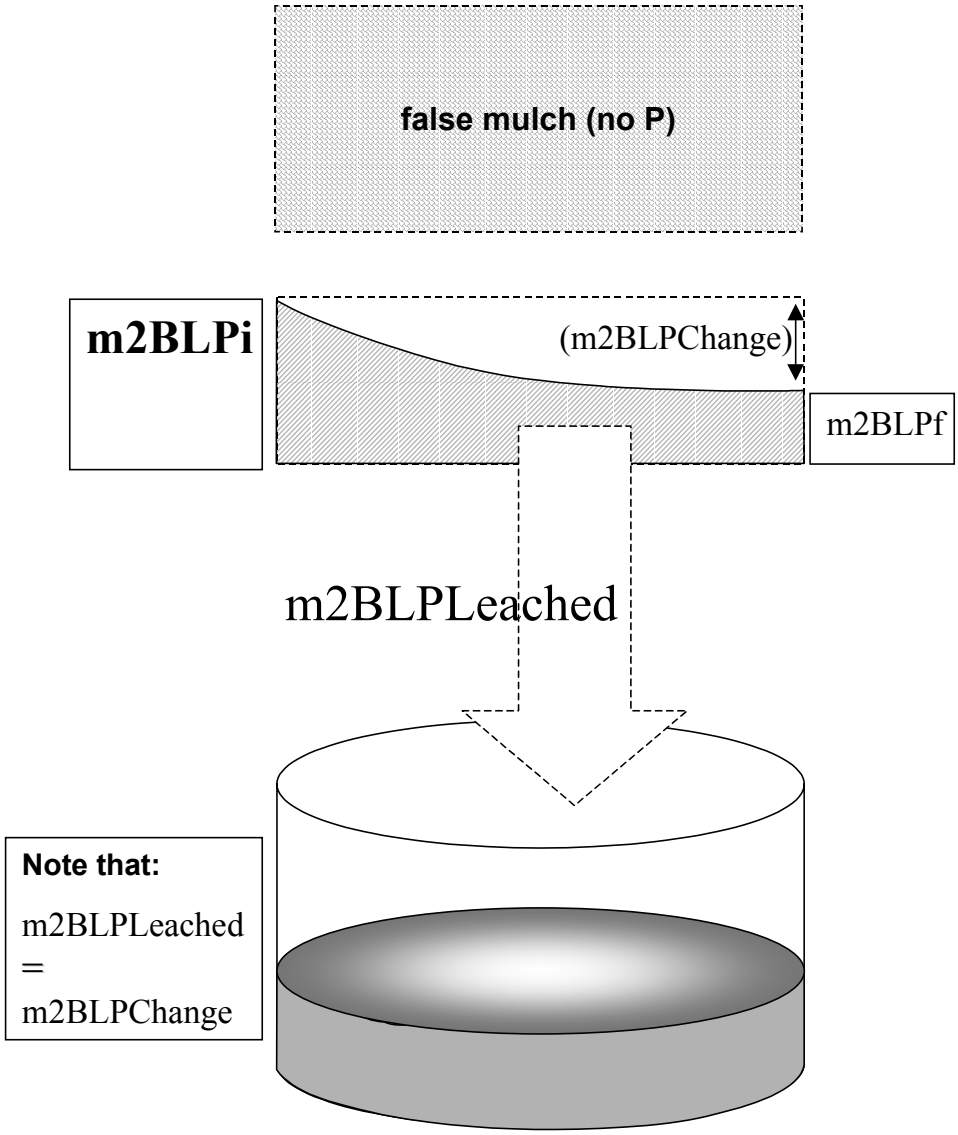


Figure 4.2: P dynamics in two-layer decomposition chambers: measured variables in a false-mulch "blank" chamber

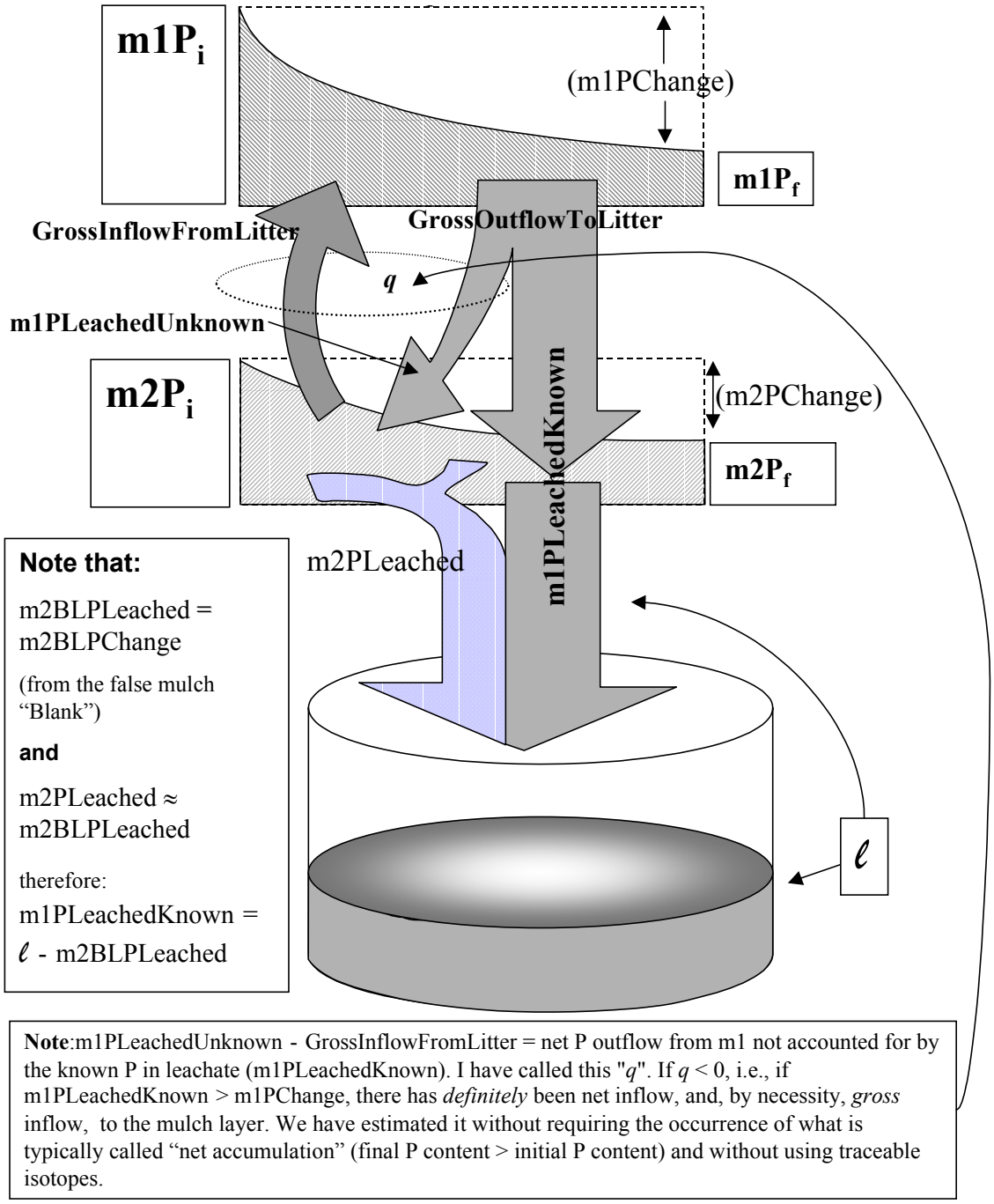


Figure 4.3: P dynamics in two-layer decomposition chambers: "actual" (but hidden) flows and measured pools in a sample chamber

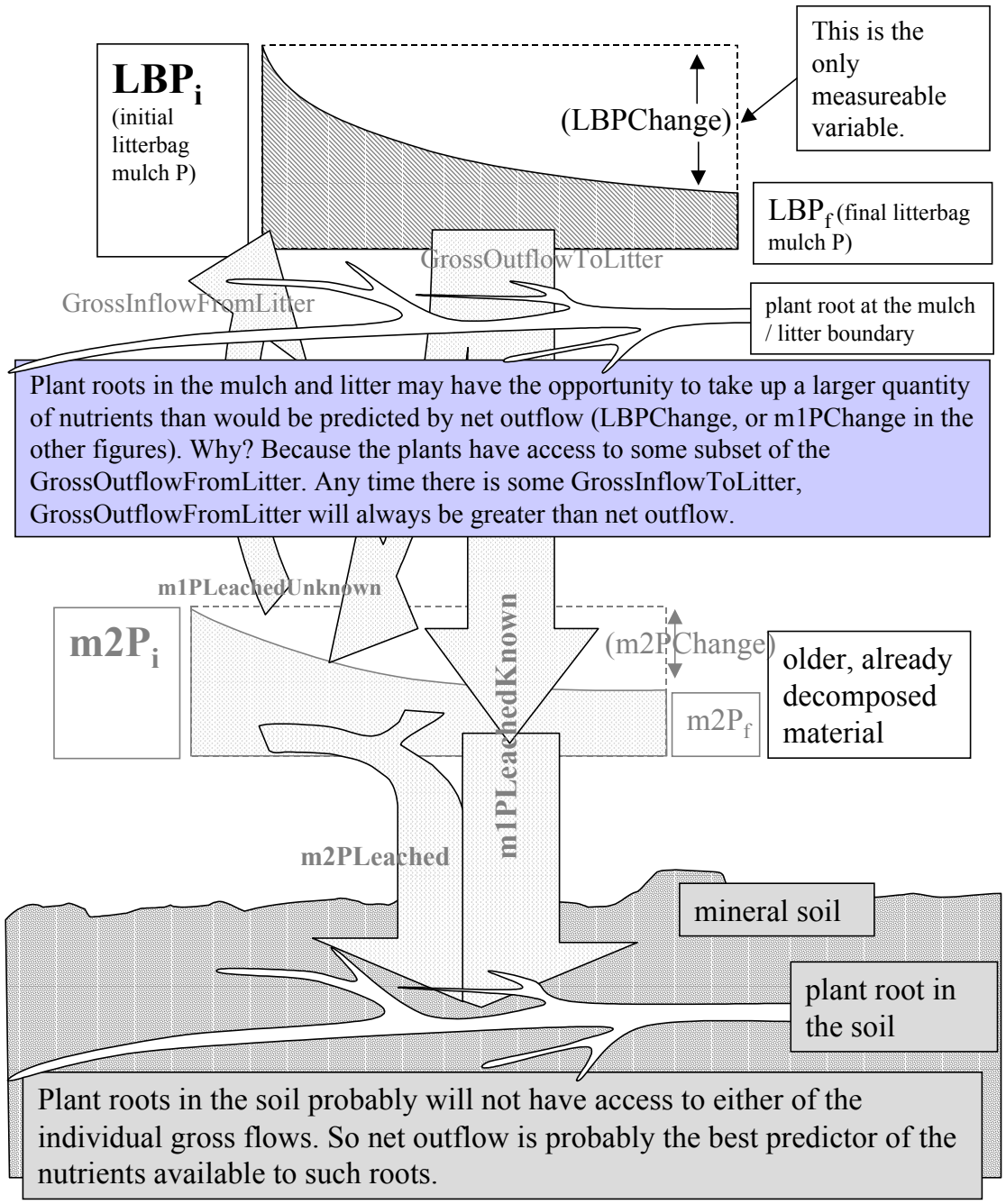


Figure 4.4: Measurable nutrient dynamics in typical litterbag litter decomposition experiments

CHAPTER 5

MATERIALS AND METHODS

5.1. Site description

This study was conducted in Costa Rica at the Las Cruces Biological Station, one of the field stations belonging to the Organization for Tropical Studies consortium. This station is located at 8° 47' N, 82° 57' W near the Panamanian border on the southern Pacific coastal range, several kilometers south of the town of San Vito de Jaba, in the Canton of Coto Brus, Puntarenas Province. The containers used in this study were placed in the Station's demonstration vegetable garden, at an altitude of approximately 1,100 meters. This site receives approximately 3-4 meters of rain annually, with a single dry season running approximately from late December until May and a rainy season for the rest of the year which reaches its peak in November. As described in Jin *et al.* 2000 (Jin et al. 2000), this area is categorized in the Holdridge climatic classification system as tropical premontane rain forest (Hartshorn 1983) and in the tropical mountain orobiome of the equatorial, humid, diurnal climatic classification scheme of Walter (1985). Mean annual temperature is 21°C. Sample materials were obtained from local farms.

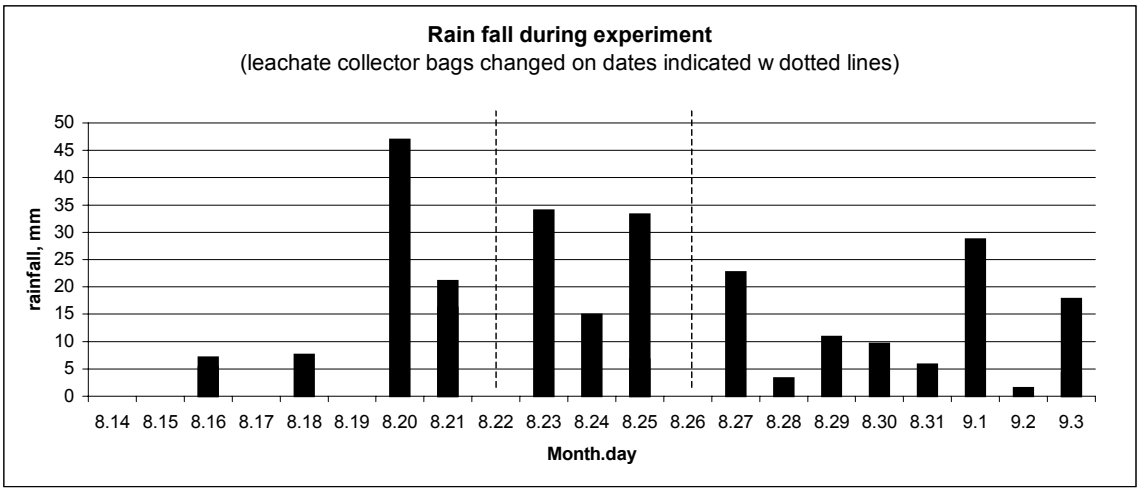


Figure 5.1: Rainfall at the Las Cruces Biological Station August 14 - September 3, 1999

5.2. Decomposition environments

5.2.1. Traditional litter bags

The traditional litter bags were made from nylon window-screening material. The pocket space for holding the litter is 20 by 20 cm, cm deep with a mesh-hole size of 1 mm^2. The height of the pocket space was kept roughly the same across the entire pocket by inserting a cardboard blank into the space while gluing together the two halves. The screening material was bleached and acid bathed prior to use. The glue used was a PVC-pipe-gluing cement. The glue strip formed a 1 cm border on two or three sides (depending on whether the packet was made from 1 or 2 pieces of material). Fresh plant material (mulch) was placed in the pocket and the extended flap approximately 7 cm long was folded back into the pocket to close it off.

5.2.2. Free-standing, two-layer decomposition chambers

5.2.2.1. General design

The two-level chambers were used in two different contexts:

a) leachate-capturing, soil-free, closed system, and

b) non-leachate capturing, placed on soil-surface, open system.

The chambers were constructed from polyethylene plastic tubs purchased from the Plasticos Modernos S.A. corporation (La Rivera, Heredia, Costa Rica). They are normally used by the Dos Pinos corporation as one gallon retail-sale ice-cream containers. The tubs used in this experiment were purchased directly from the manufacturer and had not been previously used. They were plain white, semi-translucent, and had nothing printed on them.

The tubs were 22.5 cm wide across the top; slightly narrowing to 19.6 diameter across the bottom, with a height of 21 cm. They come with secure-fitting lids.

The general idea is to create a two-layer chamber with fresh material (mulch) in the top layer which rests freely on top of old, already-decomposed material (litter) on the bottom. The litter is intended to serve as a source of decomposer biota which may attack the mulch and/or capture and retain some of the nutrients leaching from it.

It is extremely important that the top level remain unattached to the rest of the container. It must rest directly and freely on top of the litter in such a way that, as the litter continues to decompose and settle, the mulch settles down right on top of it without leaving any air gap. To meet this design requirement, I cut the tubs in half, approximately 11 cm up from the bottom. The bottom half became a 10 cm tall "narrow" tub with a closed bottom, and the top half became a 11 cm tall "wide" cylinder, open on both the top and bottom. This cylinder is eventually closed off on the bottom and serves as the lower level for holding the litter. The narrower half-tub, which had been on the bottom, will have its closed bottom cut out, replaced with nylon screening, and placed on top of the litter inside the wider container. The nylon mesh used here was the same as that used in the litterbag treatment. Because it is narrower than the cylinder, the top level rests directly and freely on the contents of the lower level.

The narrower tub has its bottom cut out, leaving an interior border approximately 1 cm in width. Using the PVC glue mentioned before, I attached a circle of the same nylon window-screening used to make the litter-decomposition bags.

The tub tops were used to make bottoms for the open-bottomed "wide" cylinders (originally the top halves of the ice-cream tubs). Four pie-shaped wedges were cut from the tops, leaving as much open space as possible without compromising the structural integrity of the disk. This cut-up top is glued to the bottom (narrower) end of the wide cylinder.

Clear plastic bag material was taped to the interior of the upper layer, folded over its edge and out far enough to cover the small gap between the walls of the upper and lower layers. The presence of this cowling ensured that no rain water could fall directly into the litter.

5.2.2.2. Leachate-capturing treatment

In order to capture nutrients leaching out of the mulch and litter, the two-layer containers described above were placed on top of 1 gallon ice-cream tubs, the same tubs which I chopped up to make the containers. The leachate was captured in plastic bags placed in the tubs. The bags were large enough so that they could be secured along the rim of the tub by rolling the top part of the bag inside out. Air holes were melted into the side of the tub to ensure that the leachate bag was free to expand within the tub as needed. Without such air holes, leachate cannot flow out of the litter and into the bag. This can ruin your experiment. Air holes were also melted into the rim of the top which had been glued on to the bottom of the lower, litter, layer. These air holes allow for the escape of air displaced from *within* the leachate bag. Again, without such holes, leachate will cease to flow and will back up in the litter, and the experiment will be ruined.

5.2.2.3. Soil-surface treatment

The soil-surface version of the two-level litter-decomposition chamber did not need any further modification. The chambers used in this

treatment were simply placed on the bare soil surface. They were surrounded by mulch so as to reduce direct radiation of the sides of the containers and to reduce the amount of exposed soil likely to splash into the chambers during rainshowers.

5.3. Plant materials

5.3.1. Material collection

5.3.1.1. Mulch

Leaf material was collected from two trees on an former coffee farm neighboring the field station. The trees had been planted in amongst the coffee bushes to provide shade and, presumably, additional nitrogen. Reference specimens are stored at the Las Cruces Biological Station. Definitive identification is pending, but my preliminary identification without floral material indicates that the trees sampled were *Inga oerstediana* (Zamora 1991).

Between collection and distribution to their respective containers and sites, mulch material was stored, loosely packed in plastic mesh grain sacks. Some air-drying of the mulch undoubtedly occurred during that time.

5.3.1.2. Litter

Litter material was collected from Finca Loma Linda, an experimental farm outside of the town Cañas Gordas on the Panamanian border, approximately 30 kilometers from the Las Cruces Field Station. This farm is the site of several other *frijol tapado*-related experiments (Rosemeyer 1990, Rosemeyer and Barrantes 1992, Rosemeyer and Gliessman 1992, Kettler 1995, Kettler 1997b, Kettler 1997a, Schlather 1998, Rosemeyer et al. 2000). The experimental plots planted with rows of *Inga* trees provided the litter material used in this experiment. As part of the other researchers' experimental protocols, operational definitions had been established for different age classes of decaying plant material. What I am calling "litter" refers to all decaying plant material already on the ground *above* but *not including* any mineral soil. This material was collected by the same field hands who have collected the material for the other researchers, thereby allowing for comparison between their work and mine. In their work, this material is referred to as "intermediate mulch".

Enough litter material was collected from the soil surface of the *Inga* plots at Finca Loma Linda to provide for the same area in my tub and litterbag treatments. Extra material was collected to allow for a "buffer" in the mixing bag when distributing litter among the different replicates.

5.3.2. Material processing for initial samples: chamber preparation

5.3.2.1. Distribution

5.3.2.1.1. Mulch

Immediately prior to distribution into the containers and sites, stems, petioles, and the winged rachises were removed from the leaflets, leaving only the leaflet body and its petiolules, so as to reduce the variability resulting from combining soft and woody material. The remaining mulch leaflet material was chopped with a paper cutter; leaflets wider than approximately ten centimeters were chopped in half along the length of the petiolule. All leaflets and half-leaflets were then cut across the petiolule at approximately every seven centimeters. The chopped mulch was returned to the woven plastic sacks and mixed.

Enough *Inga* material was collected to roughly replicate the depth of mulch material observed in the *Inga* plots in Kettler's experiments. After trying several different quantities, the best tradeoff between depth on one hand and danger of overflowing on the other was obtained at approximately 30 grams wet weight per mulch layer in the litterbasket treatments. Given that the tub layers have an area of approximately 0.05 m^2, this is equivalent to approximately 600 g / m^2 or 6,000 kg / ha. The average dry mass across the Leachate-Collector and Soil-Surface mulch layers was 25.10 grams, standard deviation: 0.22 grams. This is equivalent

to 501.94 g / m^2 and 5,019.4 kg / ha. The litterbags were designed to reproduce the typical litterbag, and therefore they did not have room for the same depth of mulch as was used in the litterbaskets. The average dry mass per litterbag was 6.81 grams with a standard deviation of 0.13 grams. The litterbags had an area of approximately 0.04 m^2, resulting in approximately 170.2 g / m^2 or 1,702 kg / ha.

Enough extra material was collected to ensure that the sample from the parent-material bag did not require scooping from the bottom of the bag where smaller material had accumulated during the mixing and distribution process.

5.3.2.1.2. Litter

Woody material larger than 3 cm in size (in any direction) was removed from the collected litter. The litter was mixed several times by hand to homogenize it before distribution into the various treatment containers and sites. Between collection and distribution, the litter was stored in open, clear plastic bags.

As with the mulch, a buffer of extra material was allowed for in the mixing and distribution bag to ensure that there was no need to scoop from the bottom of the bag for any given replicate. Approximately 120 g of litter (wet weight) was placed in each litter layer of the Leachate-Collector and Soil-Surface treatments. The average dry mass was 35.64 grams, standard deviation: 1.86 grams. This is equivalent to 712.77 g / m^2 and 7,127.7 kg / ha.

5.3.2.2. Sampling for dry/wet mass conversion and P determinations

At ten evenly spaced intervals during the process of distributing mulch and litter into treatment-layers, three samples were obtained for:

1. dry-weight/wet-weight conversion,
2. total-P determination, and
3. microbial-P determination.

1. Dry-weight/wet-weight conversion samples were placed in pre-weighed metal containers and weighed. They were dried at 60^{o}C until stable weights were obtained. The metal containers were closed prior to removal from the oven for final weighing so as to prevent the subsamples absorbing moisture from the humid air.
2. Total-P samples were placed in plastic bags and dried at 60^{o}C until stable weights were obtained so as to prevent further decomposition-related mass loss prior to total-P analysis.
3. Sufficient sample material for both microbial-P subsamples (chloroform fumigated and non-fumigated) was placed in weighed bags, the weight was recorded, and the bags closed to prevent moisture loss. These bags were then set aside to be dealt with after finishing the process of distributing all the mulch and litter to the chambers and placing the chambers in the field.

5.3.2.2.1. Microbial-P sample processing

Sample mulch material was chopped, in the bag, into pieces no larger than roughly 5cm on any side. This was done to ensure that the sample material would be able to float freely in the bicarbonate extraction solution. Litter material did not need to be chopped. The sample material was then homogenized in the bag through shaking and hand mixing. The bag, with litter in it, was reweighed and the weight recorded. Differences between this weight and the previous one were considered to be the consequence of moisture loss during the cutting process. This moisture loss was accounted for when calculating the final P concentration.

The sample material was then divided into two equal sub-samples, each of which was placed in a sealable jar and weighed. One ml of chloroform was placed in one of the jars, but not the other. Both jars were then sealed. Approximately 24 hours later, approximately 200 mls of bicarbonate extractant solution was placed in both jars. The sample/extractant ratio was kept the same across sample material types (mulch vs. litter), using approximate sample dry-weights to reduce the influence of across-treatment differences in sample material moisture.

The sample material remained submerged in the extractant for approximately 48 hours. At that point, an aliquot of the extractant was transferred to a centrifuge tube and acidified to stop the extraction process and to flocculate any suspended sample material.

5.3.3. Final sample processing

5.3.3.1. Basic processing

The mulch and litter material in each chamber layer was dumped into a pre-weighed plastic bag, weighed for total wet weight, homogenized, and divided into the same three samples as described above. The mulch layers of the two container treatments exhibited a sharp difference between the dry leaves on the surface and wetter ones underneath the surface. To reduce the effect of such heterogeneity, two wet/dry-conversion samples were taken from each replicate. The conversion ratios of these two samples were averaged, and this average was used with the wet weight of the entire layer to derive its dry-weight.

5.3.3.2. Special processing for a subset of the mulch layer samples: testing for mineral soil contamination from soil splash-in in the Soil Surface treatment

Mineral soil splashed into the mulch layers of the Soil Surface treatment containers across the anti-splash buffer zone surrounding. Much of this soil was loose and fell away from the mulch material at processing time. Removing mineral soil dried to the mulch itself, though, required more drastic measures. I decided to rub off the deposited soil from the individual leaf pieces over a piece of paper and then separate, as best as possible, the material that had dropped onto the paper into plant material

and mineral soil. A sub-experiment was set up to determine the impact of this cleaning procedure.

Because the mulch layers of the Leachate Collector treatment did not have any mineral soil splash in to them, they could be used to determine this impact. Individual replicates were split into cleaned and uncleaned halves. Any difference between them in the Leachate Collector treatment was considered to be the undesired effect of the cleaning procedure. This effect was compared to the difference between the cleaned and uncleaned replicate-halves in the Soil Surface treatment. The difference between these effects in the two treatments was used to determine the extent of the mineral soil contamination in the Soil Surface treatment. The cleaning-procedure effect was used as a correction factor on a per-unit dry-weight basis to determine the dry-mass and P-pool size as they would have been without cleaning.

Half (five) of the mulch layer replicates from each of the Soil Surface and Leachate Collector treatments were randomly selected to be part of this experiment. The decomposed mulch material in each of these replicates was split into two parts along a diameter line of the tub. One half was cleaned and the other half, not. The other five Soil Surface Mulch replicates were cleaned in their entirety. The remaining Leachate Collector replicates were processed *without* cleaning, i.e., according to the protocol used when processing the initial samples.

Because of the extended time the moist samples were exposed to the air, it was possible that their dry/wet ratios would be very different from those of the dry/wet-conversion subsamples taken at the beginning of the processing. Mass data were collected during the processing in order to determine moisture loss and generate a correction factor which would allow the samples' dry weights to be correctly determined using the original dry/wet-conversion ratios.

5.3.3.3. Special processing for a subset of the litter layer samples: testing for mineral soil contamination in the Soil Surface treatment

Because the litter layers of Soil Surface replicates were in semi-direct contact with the mineral soil through nylon mesh, it was possible that some soil had entered into the containers, adding some excess mass and potentially large quantities of total-P. Furthermore, mineral soil which splashed into the mulch layer of the replicates rolled and flowed down onto the top of the litter layer. I set up a sub-experiment to determine the impact of these sources of contamination.

The litter layer replicates were separated into three sub-layers: top, middle, and bottom. The top and bottom were assumed to be contaminated and were lumped together. Proportionally-sized samples for total-P analysis were removed from both the lumped top+bottom and the middle sub-layers. All the sub-layers were then mixed back together before

removing the dry/wet weight conversion and microbial-P analysis samples.

All replicates of the Soil Surface treatment were processed in this manner, but total-P determinations of both sub-layer groupings were made for only half of the replicates. Half of the Leachate Collector treatment replicates were processed this way to determine whether there had also been a non-contamination-related partitioning of total-P in the sub-layers.

5.3.4. Experiment timeline

All dates in 1999:

Day	Description
August	
01	Soil for the SoilSurface and LitterBag treatment plots cleared of vegetation and levelled.
02	Leaves collected from trees for mulch layers. Litter collected, mixed, and large woody material removed.
12	Mulch chopped and mixed.
14	**All treatment containers placed in the field.** Samples obtained for dry/wet mass conversion and P determinations.
15	Microbial P subsamples processed from bags into jars.
16	Chloroform added to fumigation microbial-P subsamples.
17	Bicarbonate extraction solution added to all microbial-P subsample jars.
18-19	Extractant transferred from jars to centrifuge tubes and neutralized to prevent further extraction.
22	Leachate bags have been filled by rain; collected and replaced with new ones.
24	Digestion of extractant from microbial P subsamples.
26	Leachate bags have been filled by rain; collected and replaced with new ones.
27-28	P analysis of digested extractant from microbial P subsamples.
September	
03	**Litterbags and Soil Surface treatment containers covered in the field to prevent further exposure to rain. Leachate Collector treatment containers moved under roof of laboratory porch.**

Day	Description
05	**Litterbags placed in plastic bags. Soil Surface treatment containers moved to laboratory.**
12	Litterbag processing.
14 - Oct 10	Chamber layer processing

5.4. Field plot layout

5.4.1. Free-standing treatments and blanks

All containers and litterbags were placed in the field on August 14, 1999. The containers were placed in the demonstration vegetable garden at the field station. The freestanding chambers (Leachate Collector treatment, False Mulch Blanks, and Rain Blanks) were placed on a wire mesh platform 3 meters by 1 meter raised approximately 10 cm above the soil. The platform was placed along a north-south axis, and the containers were arrayed in two, interdigitated rows along this axis so as to ensure that all containers had the same mix of exposure and protection. In order to reduce the likelihood of mulch fragments splashing into the Rain Blanks, they were placed on the opposite end of the platform from the Leachate Collector replicates, with the False Mulch Blanks in the middle. The platform was covered with plastic mesh grain sacks to prevent rain from passing through the mesh and splashing mineral soil back up into the containers. The containers were placed 25 cm in from the edge of the platform to ensure that no mineral soil splashed up from around the platform.

5.4.2. Soil-surface treatments

5.4.2.1. Soil preparation

The Litter Bag and Soil Surface replicates were placed on the soil surface several meters from the raised platform carrying the free-standing chambers. The soil in this area was turned over and leveled two weeks before placing the chambers and bags in the field. The purpose of this time gap was to allow time for the soil to settle again and reduce the influence of a sudden flush of nutrients as a result of turning-related root death and decomposition.

5.4.2.2. Litter Bag treatment

Preparing for the placement of the Litter Bag replicates, I laid down a layer of litter on the soil surface equivalent in thickness to that placed in the bottoms of the two litterbasket treatments. The Litter Bag replicates were placed on top of this layer in a checkerboard pattern such that the litter bags touched each other only at their corners. The litter bags were covered with and surrounded by mulch (i.e., the same material as that *inside* the litter bags). The litter bags were designed to occupy a fraction of the mulch layer; the entire layer was equivalent in depth to that placed in the top layer of the litterbasket treatments.

5.4.2.3. Soil Surface treatment

The Soil Surface replicate chambers were placed directly on the soil surface (i.e., there was no need to place a layer on the soil surface first because each chamber contained its own litter layer). The chambers were surrounded by layer of mulch which was intended to cover the adjacent bare soil and thereby eliminate any splashing-in of soil during the heavy afternoon rains.

Four days after placing the Soil Surface chambers into the field, water was observed pooling up around the chambers. Shallow drainage canals were dug at the margins of the plot to prevent further pooling.

5.5. Chemical analyses

5.5.1. Introduction

The entire contents of the containers were weighed for wet weight. Subsamples of plant tissue (fresh mulch and old litter) from the containers were analyzed for:

1) dry mass/wet mass conversion factor, used to determine total container Dry Mass (DM)
2) Total P (TP)
3) Chloroform-fumigated, bicarbonate-extracted P (CFBEP)
4) Bicarbonate-extracted P (BEP)

Subtracting BEP from CFBEP is supposed to provide an index of Microbial P (MP) (McLaughlin et al. 1986).

Collected leachate will be analyzed for:

1) Total volume collected

2) Organic P + inorganic P

3) maybe for inorganic P by itself

All P analyses except for the TP involve some sort of extraction followed sometimes by a digestion (for analyzing dissolved organic P) and followed always by a spectrophotometric analysis for the extracted (and digested) inorganic P.

5.5.2. Total P

TP analyses were performed at the soil-chemistry laboratories of the Centro de Investigación Agropecuaria of the Universidad de Costa Rica in San Jose. Samples were dried, ground, and underwent Kjeldahl digestions before analysis in a Flow Injection Analyzer.

5.5.3. Chloroform-fumigated, bicarbonate-extracted P

CFBEP analyses were performed in the laboratory at the Biological Station. Subsamples were sealed in jars with 2 mls of chloroform for 24 hours. All subsequent steps are identical to those carried out on BEP, as described below in Section 5.5.4.

5.5.4. Bicarbonate-extracted P

BEP analyses were performed in the laboratory at the Biological Station. Subsamples were placed in jars with a 1.5 molar sodium bicarbonate solution. Aliquots of this extractant were removed and acidified to roughly neutral in order to stop the extraction process. Subsequent processing follows the steps described below in Section 5.5.5.

5.5.5. Organic P + inorganic P

Sample solutions were centrifuged to remove suspended solids. The supernatant was then digested in a pressure cooker at 15 psi for one hour, exposed to 1.8 N H_2SO_4 and, as an oxidant, a 0.5M solution of potassium persulfate (Tiessen and Moir 1983). Digestion efficiency was determined using standards of known concentration of glycerophosphate.

5.5.6. Spectrophotometric analysis for inorganic P

Spectrophotometric analysis for inorganic P followed the ascorbic acid, molybdenum blue method described in Murphy and Riley (1962). The analyses were conducted on a portable student spectrophotometer in the laboratory at the Las Cruces Field Station.

Sample solutions were adjusted to the same pH using a p-Nitrophenol indicator. The ascorbic acid, molybdenum blue reagent was added to the samples and color was allowed to develop over a 45 minute period. Using a one cm cuvette, sample solutions were analyzed at 882 nm

and their absorption readings were compared to those of known concentrations of PO_4 (from KH_2PO_4) in solutions prepared with distilled, deionized H_2O.

5.6. Statistical analyses

For each of the variables listed earlier in Section 5.5.1, a percentage change across the sample period is calculated for each replicate. This allows for comparisons across treatments which use different quantities of the measured materials and eliminates the influence of the small variations in quantities used in each replicate of any given treatment. Percentage change was calculated in the following way:

> % change of x = [(final value of x) - (initial value of x)] / (initial value of x)

Therefore, a negative % change indicates a loss of the given substance while a positive change indicates an accumulation of that substance.

5.6.1. Treatment comparisons

Treatments were compared using the Model I Analysis of Variance (ANOVA) (Sokal and Rohlf 1995, Ihaka and Gentleman 1996). When comparing two samples, I conducted an F-test for homogeneity of

variances. If the null hypothesis of equal variance was rejected, I used Welch's approximate t-test.

The comparisons of interest are:

1) Leachate Collector mulch vs. Litter Bag mulch, and
2) if there is a significant difference between these two, compare them with Soil Surface to see if it occupies an intermediate value between them.

CHAPTER 6

RESULTS

6.1. Data included

Not all the samples collected have been analyzed, and therefore the data set is not entirely complete. The critical data sets: dry mass and total P, both initial and final, are complete. For the secondary data sets of bicarbonate-extractable P (BEP) and chloroform-fumigated BEP (CFBEP), I have a complete set of initial values, but the final values data set is half complete for each treatment, permitting statistical comparison albeit with smaller sample sizes. None of the leachate samples have been analyzed, but the critical value of total P leached over the course of the experiment can be calculated from the total P lost from the mulch and litter layers.

6.2. Comparing the free-standing, closed-system, two-layer litter-decomposition chambers to litter bags

Before assessing all the data collected from my modified litterbaskets, it is critical to determine the level of artificiality arising from their design by comparing them to litterbags, in terms of change in the mass and phosphorus pools measured. The results of *t*-test comparisons of those pool changes are reported below in Table 6.1.

Table 6.1: Comparing pool changes in my leachate-collectors vs. litterbags using *t*-tests: (A) dry mass; (B) total P; (C) chloroform-fumigated, bicarbonate-extracted P; (D) chloroform-fumigated, bicarbonate-extracted P, (one outlier removed from each treatment); (E) bicarbonate-extracted P.

	Litterbag	Leachate-Collectors
A. Dry mass; n = 10		
mean % lost	11.33%	10.09%
standard deviation	1.88%	4.02%
P (α)	0.39	
Power (1-β)	0.51	
observed difference	1.23%	
largest undetectable difference with power of 95%	3.52%	
largest undetectable difference with power of 90%	3.00%	
B. Total P; n = 10		
mean % lost	21.12%	28.65%
standard deviation	9.01%	10.61%
P (α)	0.10	
Power (1-β)	0.48	
observed difference	7.53%	
largest undetectable difference with power of 95%	15.71%	
largest undetectable difference with power of 90%	13.96%	
C. Chloroform-fumigated, bicarbonate-extracted P; n = 5		
mean % lost	14.15%	40.79%
standard deviation	26.00%	27.59%
P (α)	0.15	
Power (1-β)	0.48	
observed difference	26.63%	
largest undetectable difference with power of 95%	60.16%	
largest undetectable difference with power of 90%	52.99%	

	Litterbag	Leachate-Collectors
D. Chloroform-fumigated, bicarbonate-extracted P, (one outlier removed from each treatment): n = 4		
mean % lost	25.34%	29.94%
standard deviation	8.19%	15.18%
P (α)	0.61	
Power (1-β)	0.52	
observed difference	4.60%	
largest undetectable difference with power of 95%	17.78%	
largest undetectable difference with power of 90%	14.78%	

E. Bicarbonate-extracted P: n = 5		
mean % lost	34.78%	56.93%
standard deviation	25.76%	8.16%
P (α)	0.13	
Power (1-β)	0.50	
observed difference	22.15%	
largest undetectable difference with power of 95%	46.55%	
largest undetectable difference with power of 90%	41.16%	

6.3. Initial measurements of mulch and litter characteristics

All initial values were determined by analyzing ten samples from the source material used to fill my modified litterbaskets and litterbags. In this section reporting initial measurements, I will not be distinguishing between the Leachate-Collector and Soil-Surface treatments; rather, I will refer to them collectively as "litterbaskets".

6.3.1. Total-P

Table 6.2: Total P concentration of mulch and litter (% of dry mass)

layer	mean	standard deviation	minimum	maximum
Mulch	0.152%	0.009%	0.14%	0.17%
Litter	0.106%	0.010%	0.09%	0.12%

Applying these concentrations to the average dry mass values for each treatment, reported previously in section 5.3.2.1, the following average P absolute amounts were applied (assuming an area of 0.05 m^2 for the litterbasket levels and 0.04 m^2 for the litterbags):

Table 6.3: Total P (kg / ha) in the mulch and litter layers of the Litterbaskets and Litterbags

layer	Litterbasket	Litterbag
Mulch	7.62	2.59
Litter	7.56	no litter layer

6.3.2. Chloroform-fumigated, bicarbonate-extracted P

Table 6.4: Chloroform-fumigated, bicarbonate-extracted P (CFBEP) concentration of the mulch and litter (% of dry mass)

layer	mean	standard deviation	minimum	maximum
Mulch	0.069%	0.012%	0.052%	0.088%
Litter	0.034%	0.003%	0.028%	0.037%

Applying these concentrations to the average dry mass values for each treatment, reported previously in section 5.3.2.1, the following average P absolute amounts were applied:

Table 6.5: Chloroform-fumigated, bicarbonate-extracted P in the mulch and litter layers of the Litterbaskets and Litterbags (kg / ha)

layer	Litterbasket	Litterbag
Mulch	3.458	1.173
Litter	2.424	

6.3.3. Bicarbonate-Extractable Phosphorus

Table 6.6: Bicarbonate-extracted P (BEP) concentration of the mulch and litter (% of dry mass)

layer	mean	standard deviation	minimum	maximum
Mulch	0.069%	0.012%	0.052%	0.088%
Litter	0.020%	0.002%	0.017%	0.024%

Applying these concentrations to the average dry mass values for each treatment, reported previously in section 5.3.2.1, the following average P absolute amounts were applied:

Table 6.7: Bicarbonate-extracted P (kg / ha) in the mulch and litter layers of the Litterbaskets and Litterbags

layer	Litterbasket	Litterbag
Mulch	2.882	0.977
Litter	1.411	

6.3.4. Microbial-P

Microbial P is calculated by subtracting bicarbonate-extracted P (BEP) from chloroform-fumigated, bicarbonate-extracted P (CFBEP). Below I test to determine whether the CFBEP values are larger than the BEP values using a one-tailed, paired-sample *t*-test.

Table 6.8: Chloroform-fumigated, bicarbonate-extracted P (CFBEP) vs Bicarbonate-extracted P (BEP) in the mulch and litter as a percentage of dry mass (n = 10)

		CFBEP	BEP
Mulch			
	mean % of initial dry mass	0.069%	0.057%
	standard deviation	0.012%	0.010%
	observed difference	**0.011%**	
	***P* (*α*)**	**0.00005**	

	CFBEP	BEP
Litter		
mean % of initial dry mass	0.034%	0.020%
standard deviation	0.003%	0.002%
observed difference	**0.014%**	
***P* (*α*)**	**0.0000006**	

The two sets of values appear to be statistically different for both the mulch and litter layers. Below follows a calculation of the microbial P.

Table 6.9: Microbial P concentration in mulch and litter (% of dry mass)

layer	mean	standard deviation	minimum	maximum
Mulch	0.011%	0.005%	0.003%	0.021%
Litter	0.014%	0.004%	0.008%	0.018%

Applying these concentrations to the average dry mass values for each treatment, reported previously in section 5.3.2.1, the following average P absolute amounts were applied:

Table 6.10: Microbial P in the mulch and litter (kg / ha)

layer	Litterbasket	Litterbag
Mulch	0.576	0.195
Litter	1.013	

6.4. Mass and P pool changes

I have compared all the mulch and litter properties below for the three treatments using analysis of variance, followed by adjusted pair-wise t-test means separation. The data are tested for homogeneity of variances using Bartlett's K-squared test. The results of that test are reported if they have a *P* value less than 0.05. Comparisons of litter pools

are made using *t*-tests because there are only two treatments with litter layers.

All values in this section, unless specified otherwise, refer to the final quantity of a given pool in terms of percent of that pools initial size. In this section documenting pool-size change, I will no longer be referring to the litterbaskets collectively; rather, I will be distinguishing between all three decomposition-container treatments: Leachate-Collector, Soil-Surface, and Litterbag.

6.4.1. Dry mass remaining

Sections 6.4.1.1 and 6.4.1.2 below document the change in dry mass in the mulch and litter layers of the treatments that have them (litterbags do not have the litter layer).

6.4.1.1. Mulch layer dry mass change

Table 6.11: Dry mass remaining in the mulch (as a % of original dry mass) of the Leachate-Collectors, Soil-Surface, and Litterbag treatments. Treatments are compared with analysis of variance.

treatment	mean (%)	standard deviation	*P* (*α*)
Leachate-Collector	89.9%	4.0%	**0.02057**
Soil-Surface	92.6%	2.7%	
Litterbag	88.7%	1.9%	
n=10			

Table 6.12: Dry mass remaining in the mulch layer of the three treatments (expressed as percent of initial dry mass), means separation

***P* (*α*)** for comparisons using t tests with pooled SD, *P* values adjusted according to the Bonferroni method.		
	Litterbag	Leachate-Collector
Leachate-Collector	1.000	
Soil Surface	**0.020**	0.162

6.4.1.2. Litter layer dry mass change

Table 6.13: Dry mass remaining in the litter (as a % of original dry mass) of the Leachate-Collector and Soil-Surface treatments (n=10)

statistic	**Leachate-Collector**	**Soil-Surface**
mean % lost	94.10%	99.07%
standard deviation	6.55%	1.51%
***P* (*α*)**	**0.041**	
observed difference	**4.97%**	

6.4.2. Total P change

Sections 6.4.2.1 and 6.4.2.2 below document the change in total P in the mulch and litter layers of the treatments that have them (litterbags do not have the litter layer) over the course of my experiment.

6.4.2.1. Mulch layer total P change

Table 6.14: Total P remaining in the mulch layer of the three treatments (expressed as percent of initial total P), compared using analysis of variance

treatment	**mean (%)**	**standard deviation**	***P* (*α*)**
Leachate-Collector	79.2%	10.5%	**0.0066**
Soil-Surface	75.0%	7.7%	
Litterbag	88.8%	8.8%	
n=10			

Table 6.15: Total P remaining in the mulch layer of the three treatments (expressed as percent of initial total P), means separation

***P* (*α*)** for pairwise comparisons using *t*-tests with pooled SD, *P* values adjusted according to the Bonferroni method.		
	Litterbag	Leachate-Collector
Leachate-Collector	0.0767	
Soil-Surface	**0.0063**	0.9223

6.4.2.2. Litter layer total P change

Table 6.16: Total P remaining in the litter layer of the two treatments with litter (expressed as percent of initial P), compared with a *t*-test (n=10)

statistic	**Leachate-Collector**	**Soil-Surface**
mean % lost	90.7%	101.7%
standard deviation	10.7%	14.3%
***P* (*α*)**	0.067	
Power (1-β)	0.46	
observed difference	11.0%	
maximum undetectable difference with power of 95%	22.1%	
maximum undetectable difference with power of 90%	19.8%	

6.4.3. Chloroform-fumigated, bicarbonate-extracted P (CFBEP) change

6.4.3.1. Mulch layer CFBEP change

Table 6.17: Chloroform-fumigated, bicarbonate-extracted P (CFBEP) remaining in the mulch layer of the three treatments (expressed as percent of initial CFBEP), compared using analysis of variance

treatment	**mean (%)**	**standard deviation**	***P* (*α*)**
Leachate-Collector	59.2%	27.6%	0.1750
Soil-Surface	63.2%	9.6%	
Litterbag	85.8%	26.0%	
n=5			

6.4.3.2. Litter layer CFBEP change

Table 6.18: Chloroform-fumigated, bicarbonate-extracted P (CFBEP) remaining in the litter layer of the two treatments with litter (expressed as percent of initial CFBEP), compared with a *t*-test (n=5)

statistic	Leachate-Collector	Soil-Surface
mean % lost	98.5%	130.0%
standard deviation	45.0%	100.7%
P (α)	0.540	
Power (1-β)	0.517	
observed difference	31.6%	
maximum undetectable difference with power of 95%	109.0%	
maximum undetectable difference with power of 90%	91.5%	

6.4.4. Bicarbonate-extracted P (BEP) change

6.4.4.1. Mulch layer BEP change

Table 6.19: Bicarbonate-extracted P (BEP) remaining in the mulch layer of the three treatments (expressed as percent of initial BEP), compared using analysis of variance

treatment	mean (%)	standard deviation	*P* (α)
Leachate Collectors	43.1%	8.2%	0.0864
Soil Surface	45.2%	3.2%	
Litterbags	65.2%	25.8%	
n=5			

6.4.4.2. Litter layer BEP change

For the BEP, there are only four values for the Leachate Collector. The *P* value reported below is from the unequal sample-size t-test. To obtain the most conservative power values, I generated all four possible four-sample permutations of the five-sample Soil Surface treatment set,

t-tested these against the Leachate Collector set, and used the power analysis resulting from the t-test with the lowest *P* value (0.708).

Table 6.20: Bicarbonate-extracted P (BEP) remaining in the litter layer of the two treatments with litter (expressed as percent of initial BEP), compared with a *t*-test (n=4,5)

statistic	Leachate-Collector	Soil-Surface
mean % lost	185.0%	182.5%
standard deviation	63.6%	60.9%
P (α)	0.9609	
Power (1-β)	0.5167	
observed difference	2.5%	
maximum undetectable difference with power of 95%	78.3%	
maximum undetectable difference with power of 90%	68.2%	

6.4.5. Microbial-P change

Evaluating change in microbial P first requires determining final microbial P. As discussed previously, microbial P is calculated by subtracting BEP from CFBEP for a given sample. While these data are the result of two different processes, the variability in either may reduce the utility of the final calculated value. In order to estimate the confidence we can place in this final value, I am comparing the two sets of values (BEP and CFBEP) with a paired-sample, one-way t-test for each of the treatments. These comparisons are done in absolute terms (kg/ha), because they occur *within* replicates. The final comparison of change in microbial P will be in terms of percentage of initial microbial P, to correct for the different initial masses used in each treatment.

6.4.5.1. Mulch layer change in microbial P

6.4.5.1.1. Leachate-Collector treatment mulch layer change in microbial P

Table 6.21: Comparing chloroform-fumigated, bicarbonate-extracted P (CFBEP) vs bicarbonate-extracted P (BEP) (kg/ha) in the mulch layer of the Leachate-Collector treatment (n=5)

statistic	CFBEP	BEP
mean	2.05	1.24
standard deviation	0.96	0.22
P (α)	0.0965	
Power (1-β)	0.7728	
observed difference	0.813	
maximum undetectable difference with power of 95%	1.18	
maximum undetectable difference with power of 90%	1.03	

6.4.5.1.2. Litterbag treatment mulch layer chan:ge in microbial P

Table 6.22: Comparing chloroform-fumigated, bicarbonate-extracted P (CFBEP) vs bicarbonate-extracted P (BEP) (kg/ha) in the mulch layer of the Litterbag treatment (n=5)

statistic	CFBEP	BEP
mean	1.006	0.638
standard deviation	0.329	0.268
observed difference	0.368	
P (α)	**0.0004**	

6.4.5.1.3. Soil-Surface treatment mulch layer change in microbial P

Table 6.23: Comparing chloroform-fumigated, bicarbonate-extracted P (CFBEP) vs bicarbonate-extracted P (BEP) (kg/ha) in the mulch layer of the Soil-Surface treatment (n=5)

statistic	CFBEP	BEP
mean	2.19	1.31
standard deviation	0.33	0.10
observed difference	0.88	
P (α)	**0.0027**	

6.4.5.1.4. Mulch layer microbial P remaining, compared across treatments

Having determined that the final CFBEP and BEP values are, for the most part, statistically significantly different, I will calculate final microbial P and use that to calculate percent of initial microbial P remaining. I will compare these values across treatments with an analysis of variance.

Table 6.24: Microbial P remaining in the mulch (as a % of original microbial P) of the three treatments, compared with analysis of variance.

treatment	mean (%)	standard deviation	*P* (α)
Leachate-Collector	81.5%	228.2%	0.5677
Soil-Surface	164.5%	86.4%	
Litterbag	171.2%	60.3%	
n=5			

6.4.5.2. Litter layer change in microbial P

6.4.5.2.1. Leachate-Collector treatment litter layer change in microbial P

Table 6.25: Comparing chloroform-fumigated, bicarbonate-extracted P (CFBEP) vs bicarbonate-extracted P (BEP) (kg/ha) in the litter layer of the Leachate-Collector treatment (n=4)

statistic	CFBEP	BEP
mean	2.55	2.64
standard deviation	1.25	0.79
P (α)	0.901	
Power (1-β)	0.506	
observed difference	0.096	
maximum undetectable difference with power of 95%	1.21	
maximum undetectable difference with power of 90%	0.96	

6.4.5.2.2. Soil-Surface treatment litter layer change in microbial P

Table 6.26: Comparing chloroform-fumigated, bicarbonate-extracted P (CFBEP) vs bicarbonate-extracted P (BEP) (kg/ha) in the litter layer of the Soil-Surface treatment (n=5)

statistic	CFBEP	BEP
mean	3.05	2.57
standard deviation	2.33	0.83
P (α)	0.675	
Power (1-β)	0.514	
observed difference	0.482	
maximum undetectable difference with power of 95%	2.19	
maximum undetectable difference with power of 90%	1.80	

6.4.5.2.3. Litter-layer microbial P remaining, compared across treatments

The final CFBEP and BEP values for the litter layer have much more variability than those for the mulch layer, and as a consequence, they are not testing as significantly different from each other as the mulch layer values were. I will go ahead and calculate microbial P for the treatments, and then calculate microbial P remaining, but the meaning of these values is undoubtedly suspect.

Table 6.27: Microbial P remaining in the litter layer (as a % of original microbial P) of the two treatments with litter, compared with a *t*-test.

treatment	mean (%)	standard deviation	*P* (*α*)
Leachate-Collector	-27.0%*	128.3%	0.8353
Soil-Surface	6.5%	312.5%	
n=4, 5			

*Note: This value is negative because the bicarbonate-extracted P values for the Leachate-Collector treatment were larger than the chloroform-fumigation, bicarbonate-extracted P values. That this value can be negative underscores the fact that this method of measuring microbial P provides values that are *indices*, measuring some (in this case, unknown) percentage of the actual microbial P, and that these indices are subject to measurement error and sample variability. Negative change in these microbial P values has no ecological significance or meaning. If microbial P had *decreased*, the percentage reported would be less than 100 but greater than zero. If it had increased, the percentage reported would have been greater than 100. There is no way for a change in the *true* microbial P pool to have resulted in a *negative* percentage remaining.

6.5. Closed-system litterbasket model calculation: "Fake-mulch" litter P lost vs. Leachate-Collector litter P lost

In order to calculate the variable I am calling m1PLeachedKnown, I need to determine the amount of P leached from the litter layer of the

Leachate-Collector containers. There is no way for me to do this using the Leachate-Collector litter layer data by itself. I must use the P lost from the "Fake-mulch" blanks as an approximation of that variable (see section 4.3 for a more detailed discussion of this matter). My intention was to average the P lost from those blanks, and subtract it from the P change in the litter layers of the Leachate-Collector containers. I had not intended to compare the two statistically. However, it turned out that the fake-mulch blanks played an important role in answering the central questions of this research, so now I want to see if it really is appropriate to use them in the way that I had intended. Towards that end, I will compare P change in the litter layer of the Fake-Mulch Blanks to the P change in the litter layer of the Leachate-Collectors.

Table 6.28: Total P lost from the litter layer (as kg/ha) of the Leachate-Collector treatment and Fake-Mulch Blank quasi-treatment, compared with a *t*-test.

treatment	mean P lost (kg/ha)	standard deviation	*P* (*α*)
Leachate-Collector	0.73	0.82	0.3788
Fake-Mulch Blank	1.13	0.67	
n=10, 4			

Table 6.29: Total P lost in the litter layer (as a % of original total P) of the Leachate-Collector treatment and Fake-Mulch Blank quasi-treatment, compared with a *t*-test.

treatment	mean P lost (%)	standard deviation	*P* (*α*)
Leachate-Collector	9.3%	10.7%	0.2153
Fake-Mulch Blank	17.8%	10.2%	
n=10, 4			

6.6. P release from mulch

6.6.1. Leachate P

To calculate the quantity of P leached from the fresh and old plant material in the Leachate-Collector replicates, I simply added the P lost from the upper layer of fresh material to the amount of P lost from the lower layer of old material, according to equation 4.1 (P leached = mulch P lost + litter P lost).

In the case of my leachate collectors, I have dry mass and [P] values for each individual replicate at the final sampling time, and total wet mass at the start of the experiment. Initial [P] and dry/wet conversion factors, however, were determined for samples taken from the source material at regular (recorded) intervals during the process of filling the replicate containers. The number of samples and the number of replicates was the same (10). Therefore, there are three ways to assign the values of initial dry mass (dm) and [P] to any given replicate:

1) "Mean": calculate the mean [P] and dry/wet conversion factor and use these values to calculate $P_{initial}$ for each replicate. This approach generates a single mean.
2) "Processing order": to each replicate, assign the [P] and dry/wet conversion factor from the sample obtained immediately before processing that replicate. This approach generates a single mean.

3) "Bootstrapping": randomly select, with replacement, the values obtained from the sample set to generate virtual, or bootstrapped, sets of observations. This approach generates as many means as are desired. This collection of means can be described with a frequency distribution

Neither of these appears logically more appropriate than the other. However, choosing one or the other may lead to different conclusions and interpretations of the data. Therefore, it is important to situate them in a broader context of all possible but reasonable values. Towards this end, I have calculated a range of final values based on bootstrapped sets of values. These virtual data sets were generated by randomly selecting (with replacement) the appropriate number of values from the set of observed values.

Table 6.30: P leached from both the mulch and litter of the Leachate-Collector containers, comparison of means calculated according to three different methods, reported as percentage of initial total P placed in the containers

Method of calculating the mean P leached from the mulch and litter, combined	**mean (% of initial P)**	**standard deviation**
10,000 bootstrapped sets of 10 observations, generated by randomly selecting, with replacement, from the 10 observed initial P concentrations	18.8%	1.3%
using the mean of the observed initial P concentrations	18.5%	6.3%
using the initial P concentrations from the subsamples collected immediately before filling the container	17.9%	9.5%

Table 6.31: P leached from each of the layers of the Leachate-Collector treatment containers, reported in absolute terms (kg/ha). The initial total P of each layer was calculated using the mean of the observed initial P concentrations for the given layer.

Leachate-Collector layers	mean (kg P / ha)	standard deviation
mulch	2.18	0.81
litter	0.73	0.82
total: mulch + litter	2.91	1.06

CHAPTER 7

DISCUSSION AND CONCLUSIONS

7.1. Comparing closed-system litterbaskets to traditional litterbags

In terms of mass and total-P lost, the difference in the decomposition process occurring in these two environments did not differ statistically, though the change in P content was closer to statistical significance than the change in dry mass, with the litterbags losing less P than the litterbaskets (Table 6.1). I suspect that this difference was due more to a mistaken design decision on my part than on an inherent difference between the two decomposition environments: By deciding to use litterbags roughly similar in shape and size to those used in other experiments, I forced myself to have to place the litterbags *under* other fresh *Inga* material. In this sense, the comparison I have set up is not a fair one. The fair comparison would be between the *middle* layer of the mulch in the litterbaskets with the litterbags. I could have avoided this problem by using litterbags more similar in dimensions (and mulch content) to the litterbasket mulch layer container.

The initial comparisons of the two treatments in terms of chloroform-fumigated, bicarbonate-extracted P (CFBEP) and bicarbonate-

extracted P (BEP) indicate close to statistically different *P* values as a consequence of extremely different means. However, both pools seem to have outliers that dramatically influence the means of the small data sets. Removing these outliers greatly reduces the *P* value (e.g., compare Table 6.1.C to Table 6.1.D).

Though my closed-system litterbaskets undoubtedly add all sorts of artifacts and biases, this initial analysis indicates that the decomposition processes occurring in them are comparable to those occurring in standard litterbags during the timeframe I have measured (three weeks). If further study of the issues I have raised in this thesis is warranted, I suspect that these type of container have the potential to provide useful insight into the decomposition process.

7.1.1. Soil-Surface treatment as an intermediate level of artificiality

Anticipating severe differences in decomposition processes between leachate-collectors and litterbags, I added another treatment, the "Soil-Surface" treatment, to the study, which I hoped would provide intermediate values that would help clarify which components of artificiality were playing a larger role ((a) lack of soil or (b) plastic-tub microenvironment) (see section 4.3 for a more detailed discussion of this design decision).

Because the leachate-collectors and litterbags did not differ all that significantly, the Soil-Surface treatment is not needed for its originally

intended purpose. However, its additional data may provide further insight into the decomposition process. To assess the relative importance of the two components of artificiality, I analyzed the dry mass remaining and P remaining data with the treatments grouped according to the presence or absence of the artificiality component:

treatment	**absence-of-soil**	**plastic-tub**
Litterbag	0	0
Soil-Surface	0	1
Leachate-Collector	1	1

pool % remaining = soil * tub

Pool measured artificiality	**mean (% remaining)**	**standard deviation**	***P* (*α*)**
Dry mass remaining (%)			
soil-absent	89.9	4.0	0.532
soil-present	90.6	3.0	
in-tub	91.3	3.6	**0.007**
not-in-tub	88.7	1.9	
Total P remaining (%)			
soil-absent	79.2	10.5	0.452
soil-present	81.9	10.7	
in-tub	77.1	9.2	**0.002**
not-in-tub	88.8	8.8	

There is one case in which the Soil-Surface litterbaskets behaved very differently than I expected: dry mass remaining (see Table 6.11), in which the litterbags and leachate-collectors did *not* differ significantly while the soil-surface litterbaskets *did* differ from the litterbags. Rather than having an intermediate value, the soil-surface treatment had an *extreme* value. I cannot imagine why this might have been the case.

In the case of total P remaining (Table 6.15), the Soil-Surface treatment again showed an extreme rather than intermediate value. However, in this comparison, the leachate-collectors were close to being statistically different from the litterbags as well. Therefore, it may be more appropriate to conclude that the litterbasket treatments (Leachate-Collector and Soil-Surface) were more similar to each other than they were to the litterbag treatment. This may lend support to the possibility that the "plastic tub microenvironment" was a more important source of artificiality in this study than the "lack of soil" factor. This possibility appears to be further corroborated by the cross-treatment analyses of variance for chloroform-fumigated, bicarbonate-extracted P (CFBEP) and bicarbonate-extracted P (BEP) (Table 6.17 and Table 6.19, respectively). Though none of these analyses showed statistically significant differences, they came close (which is remarkable considering the variability of the small sample sets), and in both cases, the two litterbasket treatments were more similar to each other than they were to the litterbags.

These results indicate that using leachate-collectors without soil is plausible but that the litterbasket design needs to be improved with respect to reducing microenvironmental biases.

7.2. Comparison to decomposition rates in other studies

Palm and Sanchez (Palm and Sanchez 1990) report mass and P losses for surface-applied litterbags of *Inga edulis* of approximately 15% and 5%, respectively, at week 4. The P loss had *decreased* (i.e., net P in the litter *increased*) from week 2 of the study, in which they reported a P loss of approximately 20%. In this study, I report mass losses of approximately 7-11% at week three (Table 6.11) and total P losses of 12-15% (Table 6.14). The similarity of these results suggests that the decomposition processes occurring in my litterbaskets and litterbags are not obviously abnormal.

7.3. Leached P

In Table 6.31, I report that the *Inga* mulch and litter, combined, leached approximately 2.91 kg P / ha over the course of my three-week study period. Kettler reports bean seed P yields of approximately 6 kg / ha after a full three month growing season (Figure 2.2), and approximately 2 kg P / ha of bean plant biomass after seven weeks (Figure 2.3) (excluding *Calliandra* and *Calliandra*-mixture treatments). Thus it seems plausible that bean plants could make use of the amount of P I have reported leaching during this study.

7.4. Gross inflow and outflow constrained estimate ranges

7.4.1. Hidden outflows? Comparing m1PChange to m1PLeachedKnown

One of the central purposes of this experiment was to determine whether it would be possible for me to use my modified litterbaskets to isolate any of the possible gross flows of P into and out of the mulch layer that would otherwise be obscured in a traditional litterbag decomposition study. To do this, I proposed to compare two values of P content change in the mulch layer of the litterbaskets: (1) change in total P over time (m1PChange), and (2) the known amount of P to have leached from the mulch layer (m1PLeachedKnown). Previously, in equation 4.4, I described this comparison. The former, change in nutrient content, is the traditional measurement and is easy to obtain. The latter requires measuring several other variables (each with its own sources of error), and combining them into a calculated variable, as described in equation 4.6. My calculated variable may be both greater than or less than m1PChange, and either of those outcomes would be interesting. However, the possibility of both happening makes it difficult to compare m1PChange and m1PLeachedKnown using the standard *t*-test: the means may be the same even though the distributions are different, and it is this difference in distributions that is of interest. In order to compare these two variables, therefore, I subtracted one from the other. The distribution of the outcomes of that operation should be normal if the two variables

have similar distributions. If they have different distributions, the outcomes should *not* be normal. I then tested those outcomes using the Shapiro-Wilk W test for normality. I ran this test on the 10,000 bootstrapped datasets, obtaining a *P* value for each which, if below 0.05, indicates non-normality. Out of these 10,000 tests, only four percent showed such non-normality, indicating that I was not able to gain any extra information through the use of my closed-system litterbaskets.

7.4.2. The validity of using the Fake-Mulch-Blank data

The calculation of m1PLeachedKnown is dependent on determining the "true" amount of P leached from the litter layer, i.e., the change in P of the litter layer *without* the inflow of P from the mulch layer above. I used the Fake-Mulch-Blanks to provide an estimate of this value, however, because I did not realize the importance that the Fake-Mulch-Blanks would play in the final analysis, I did not make a full treatment's worth of them (in fact, I did not have enough litter material to do so). The variability of the small set of Fake-Mulch-Blank data may, in fact, make them useless for the role I had intended. The results of the *t*-test presented in Table 6.28 and Table 6.29 show that total P change in the litter layers of the Fake-Mulch-Blanks vs. the Leachate-Collectors were not statistically different. Without different values here, there is no way to obtain a difference between m1PChange and m1PLeachedKnown. The fact that the P change in the litter layers was not statistically different could be the result of either (1) variability/sample size problems and/or (2) no

appreciable amount of P being added to the litter layer from the mulch layer. By simulating differences of different magnitudes and with different degrees of variability in the litter-layer comparisons, it should be possible to determine the sensitivity of my model to the first problem, and, therefore, by extension, the potential for observing actual differences between m1PChange and m1PLeachedKnown, if they exist, in an experiment with more Fake-Mulch-Blanks.

CHAPTER 8

FURTHER RESEARCH

Though I was not able to isolate the gross flows that I had set out to observe, we still know that they are out there. More sophisticated approaches are necessary for documenting them and their mechanism. Below I present some suggestions for those who hope to do so.

8.1. Ways to have improved this experiment

8.1.1. More Fake-Mulch Blanks

The most obvious change that would have improved the utility of this experiment would have been to increase the number of Fake-Mulch Blanks so that instead of quasi-treatment "blanks", they could have been dealt with as a treatment unto themselves.

8.1.2. More mulch-like fake mulch

The fake mulch I used, cut up pieces of woven grain-sack, was better than some fake mulches, but it certainly was not ideal. Because it was plastic, its thermal properties were very different from those of some plant material. Ideally, whatever material is chosen should

1) have the thermal properties of plant material,

2) impede the flow of rain water no more or no less than the plant material being studied, and

3) be chemically inert.

Finding just such a material may be very difficult. I suspect most materials will meet only two of the three criteria above. The plastic I used met the latter two (criterion three better than two, though) at the expense of the first one.

8.1.3. Improving the Leachate-Collector vs. Litterbag comparison

There are any number of ways to improve the Leachate-Collector vs. Litterbag comparison, most having to do with reducing the unnecessary differences between the two decomposition environments. Specifically, the litterbags should contain as much mulch as the Leachate-Collector mulch layers. In fact, they should have exactly the same dimensions; they should basically be mesh-walled versions of the Leachate-Collectors. Having the same dimensions, the litterbags can extend through the entire "mulch profile" from the open-air-mulch boundary to the litter-mulch boundary just as was the case in the Leachate-Collectors.

8.1.4. Reducing the "plastic tub" artificiality factor

As mentioned previously in section 7.1.1, the "plastic-tub microenvironment" factor seems to have been a greater source of

artificiality (defined as "difference from litterbags") than the lack of soil in the Leachate-Collectors. One obvious way of reducing this effect would be to bury the leachate-collecting layer of the Leachate-Collectors, leaving the litter and mulch layers above the soil surface, surrounded by non-contained mulch and litter, as had been the Soil-Surface containers.

8.1.5. Reducing variability in the litterbasket measurements

I had hoped to increase the strength of my analysis, relative to other studies, by reducing the variability in my treatments through the use of relatively large masses of plant material for my mulch and litter layers. Because the materials in these layers were mixed before I subsampled from them for chemical analyses, I had assumed that the variability *within* my samples would be small. However, as can be seen in Table 6.11, the standard deviations for the two litterbasket treatments were larger than that for the litterbag treatment. I suspect that the litterbasket layers were so large relative to my subsamples that no amount of mixing would have overcome their internal heterogeneity. Because the litterbags contained so much less plant mass, the subsamples I collected for chemical analyses were very large relative to the total mass used in the litterbag. Increasing the subsample/replicate size ratio for the litterbaskets may go a long way towards reducing the variability within the Leachate-Collector and Soil-Surface treatments.

8.1.6. Leachate nutrient analyses

I did not anticipate analyzing the leachates for anything other than total P. While this increases convenience by allowing for long-term sample storage, conducting a more detailed analysis on the leachates would allow for a better understanding of the processes at work in the Leachate-Collectors. Specifically, separating leachate nutrients into dissolved and particulate pools should allow the researcher to discriminate between nutrients entering in leachate as opposed to those entering as a result of simply falling through the mesh in bulk form. Furthermore, separating inorganic and organic P will be important for determining the eventual fate of the P in question, given the assumption that most inorganic P entering the soil solution will be adsorbed rapidly by the mineral soil.

8.2. Testing for the importance of gross flows

8.2.1. Plants as bioassays

If my experiment had, in fact, documented the gross flows I was hoping to find, the obvious next experiment would have been to determine their importance, if any, in plant nutrition. Such an experiment may still be worth doing even without first obtaining evidence for "hidden" gross flows. Such an experiment could have three treatments:

1) leachate-collector-type litterbasket,
2) litterbag-type litterbasket, and
3) plants growing in the same mulch and litter as is used in treatments one and two.

The question would be: which of the first two treatments provides variables that are better for predicting the observed nutrient uptake in the plant treatment.

8.3. Testing for gross flows into and out of fresh plant material: traceable isotopes?

It is clear to me now that I tried to accomplish too many goals simultaneously, and as a consequence, did not accomplish any one of them particularly well. Specifically, I should not have tried to simultaneously

1) test a new method looking for potentially difficult-to-find flows while also
2) limiting myself to using low-tech tools.

It would have been more appropriate to separate the process into two stages:

1) isolate and determine the significance of the potentially difficult-to-find flows using the best tools available for the job (e.g., traceable isotopes), and *then*, if warranted,
2) use the findings from stage one to develop methods for use in low-tech research environments.

8.4. Recommendations for future decomposition research

The conclusions of my literature review and conceptual analysis of litter decomposition research are that:

1) The significance of Berg's 1988 observation of simultaneous N outflows from and inflows to decomposing surface needle litter has not been fully appreciated, partly due to misleadingly defined terms (specifically, the word "net" in "net mineralization" and "net immobilization") within the field.
2) Berg's 1988 results demonstrate *conclusively* that it is not safe to assume that plant-available nutrients are directly determinable by measuring changes in litter nutrient content. (It was never *safe* to assume this, but it was and continues to be assumed without testing.) Therefore, it is necessary to determine the conditions under which it is or is not safe to assume this relationship.
3) This relationship has been and continues to be assumed because it is true for the contexts in which it was originally posited and continues to be tested: plant materials *incorporated* into the soil. The implicit assumption in such cases is that the plant materials (and their decomposers) are in continuous contact with a supply of nutrients from the soil solution. This assumption appears to have been expressed explicitly only in Aber and Melillo 1980 (Aber and Melillo 1980) and largely ignored since then.

4) Few, if any, appear to have explicitly set out to study decomposition processes and nutrient dynamics in cases where this assumption is *not* met. I posit that Berg's 1988 results are a consequence of this assumption not being met. Furthermore, I posit that under such conditions (surface-applied plant material with only partial contact with the soil solution), litter *physical structure* will play a critical role in determining nutrient release dynamics, perhaps even more important than that played by non-structural litter chemistry. Specifically, structural characteristics which in the "incorporated litter" research are considered to slow nutrient release (e.g., high C and lignin contents) may actually *speed* nutrient release from surface litters lacking access to the soil solution. This countervailing influence of the same properties under different conditions may help explain why litters with *intermediate* "plant residue quality index" values (i.e., plant residue *chemical* quality) were worse nutrient providers than those with the *lowest* index scores (Tian et al. 1995).

Based on these conclusions, I make the following recommendations:

8.4.1. Clear categorization of methods

Too many papers, reviewing results of other decomposition studies, mix together data from incorporated and surface-applied litter studies. I would argue that, until proven otherwise, it is not safe to assume that the

nutrient dynamics and mechanisms in these two contexts are comparable. More effort needs to be made to distinguish between these two types of studies, e.g., through more informative titles that clearly specify the location of the litter being studied.

8.4.2. "Litter physics"

Almost without exception, discussions of "litter quality" (i.e., the ability of plant material to serve as a source of nutrients for plant uptake) has been focussed on litter chemistry (Cadisch and Giller 1997). I recommend that some studies be conducted to test my hypothesis that litter structure may be more important in the case of surface-applied litter. Specifically, I would recommend an approach that categorizes and quantifies litter "packing behavior" and how it influences:

1) moisture retention,
2) gas exchange,
3) heat exchange, and, importantly
4) variation in the above properties.

These properties, in turn, will affect the behavior of resident decomposers and roots, sometimes equivalently, sometimes with opposite effect (e.g., high moisture variability may increase nutrient release from dying microbes and swelling litter material, but may reduce nutrient uptake capacity of embedded roots).

As recommended previously in section 8.2.1, experiments could be conducted using plants as bioassays for the importance of litter structural properties. Ideally, treatments would be used which:

1) controlled for litter chemistry but varied structural properties, and
2) controlled for structural properties while varying litter chemistries,

comparing both sets of above treatments with:

3) plant nutrient uptake from the different litters used

8.4.2.1. Litter physics as an "emergent property"

The structural properties of plant litter are macro-scale expressions of litter chemistry, and therefore, my recommendation to study structure *vs*. chemistry, is, on the face of it, conceptually impossible. However, I would posit that the causal chain linking litter chemistry to litter structure is far too complicated to be captured easily and consistently by a chemical resource quality index (Tian et al. 1995). Therefore, I would recommend focussing on structure as a macro-level property easily measured with macro-scale tools. Once the relationships between structure and nutrient-dynamics have been established (if in fact they exist), then the relationship between chemistry and structure can be explored, if useful. For the moment, taking this purely pragmatic wholistic approach of

ignoring the underlying causal layer will, I suspect, actually *improve* explanatory efficiency (results explained per resources invested).

8.5. Socio-Economic Limitations on Application of Mulch Management in Coto Brus and the rest of Central America

8.5.1. Land ownership, rental, and land-use decision-makers

The vast majority of farmers I met using the *frijol tapado* system grew their beans on land rented from other landowners. The rental payment was usually in the form of a percentage of the yield from the plots used. It was not typical for these farmers to return to the same plots year after year. Rather, plots became available when landowners decided to change their usage of a given parcel and needed for it to be cleared. By allowing farmers to come in and grow beans on their land, they get their land cleared, receive food, and provide a community service. It is appears unlikely, then, that:

1) the *frijol tapado* system ever really runs into nutrient limitations as a result of excessively reduced fallow periods,
2) that farmers who move from one plot to another from year to year would have any interest in investing in any given plot in the form of planting improved-mulch-providing trees, and
3) that improving yields from this system would actually result in less pressure on non-farmed areas.

However, many farmers did explain to me that they had to travel long distances to find good plots for practicing the *frijol tapado* system. Time taken to travel back and forth to the plots is less time they can spend earning money from the coffee harvest. According to these farmers, good-quality nearby plots are lacking because they have been farmed to infertility (under what system is not clear). Farmers might actually be willing to implement improved-fallow *frijol tapado* systems on their own land if it is presented as a way of reducing the need to travel to distant locations. Furthermore, landowners might be able charge higher rents on improved-fallow plots in better locations, thereby creating an incentive for adopting this modification. All of these observations are based on informal interviews and need to be studied systematically.

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APPENDIX

SAMPLE QUALITY CONTROL TESTS

1.1. Testing for soil contamination of litter layer in soil-surface treatment

There was no statistically significant difference in [P] between the middle sublayer and the combined top+bottom sublayers of the Leachate Collector treatment (t-test comparison of means of paired samples: P = 0.749). This indicates that there was no *non*-contamination-induced difference between the sublayer groupings. Removing this possibility allows us to consider the results for the SS treatment to be indicative of the presence (or absence) of soil contamination alone.

There was no contamination-induced difference in [P] between the middle sublayer and the combined top+bottom sublayers of the Soil Surface treatment (t-test comparison of means of paired samples: P = 0.587).

(n=5)	mean	stnd dev
Leachate Collector		
top+bottom	0.102	0.008
middle	0.104	0.009
Soil Surface		

(n=5)	mean	stnd dev
top+bottom	0.108	0.023
middle	0.112	0.011

Each replicate which was separated into these sublayer groupings has two [P] values. As a result, the total-P for the entire replicate can be calculated in the following two ways:

(a) "averaged":

consider the [P] values for both sublayer groupings as if they were obtained from two subsamples taken from a single, whole, sample. In this case, the [P] values should be averaged and then multiplied by the dry mass of the whole sample, or

(b) "combined":

consider the [P] values for both sublayer groupings as if they were obtained from two separate samples. Calculate total-P values for each half ([P] of the half * dry mass of the half), and combine these values to derive a total-P value for the entire

replicate. (This is an average that is weighted by the mass of the subsample from which each value was obtained.)

By default, we have used the "averaged" version of the data when conducting the subsequent statistical analyses. If and when the "combined" version produces a different statistical outcome, we will present its P values.

1.2. Testing for soil contamination of mulch layer due to soil splash-in

There was a difference in [P] between the cleaned and uncleaned halves of replicates in the Soil Surface treatment (t-test of means of paired samples; P =0.016). This difference could indicate that:

(a) the cleaning process removed P added to the mulch as a consequence of soil splash-in,

and/or

(b) the cleaning process removed P that was part of the mulch to begin with.

The strength of this second factor was determined by performing the same cleaning procedure on a subset of the Leachate Collector replicates, none of which had been exposed to any soil splash-in. There was no difference in [P] between the cleaned and uncleaned halves of replicates in the Leachate Collector treatment (t-test of means of paired samples; P = 0.815). Therefore, we can conclude that the cleaning procedure did not remove P which was native to the mulch.

(n=5)	mean	stnd dev
Soil Surface		
uncleaned	0.114	0.005
cleaned	0.106	0.009
Leachate Collector		
uncleaned	0.118	0.013
cleaned	0.116	0.011

For the Soil Surface treatment, statistical analysis will be performed on the data from the cleaned replicate halves, for those replicates which were split. The remaining replicates, which were not split, were all cleaned. Therefore "cleaned" data are available for the entire treatment.

For the Leachate Collector treatment, the cleaned and uncleaned halves of split replicates are statistically identical. Therefore, there are a variety of ways of treating these data:

(c) "averaged":

consider the [P] values for both halves as if they were obtained from two subsamples taken from a single, whole, sample. In this case, the [P] values should be averaged, or

(d) "combined":

consider the [P] values for both halves as if they were obtained from two separate samples. Calculate total-P values for each half ([P] of the half * dry mass of the half), and combine these values to derive a total-P value for the entire replicate. (This is an average that is weighted by the mass of the subsample from which each value was obtained.) Or,

(e) "ignored":

ignore the cleaned [P] value altogether. Use the uncleaned [P] value to determine the total-P for the whole replicate.

By default, we have used the "averaged" version of the data when conducting the subsequent statistical analyses. If and when the different versions result in different statistical outcomes, we will present the P

values for all the versions, which will be labeled: averaged, combined, and ignored, respectively.

1.3. Testing for effect of time lag of sample processing on measured variables

We conducted regression analyses to determine whether there was a relationship between %P lost (absolute P lost/initial absolute P) and sample processing delay (days elapsed since first sample was processed). A statistically significant relationship was found in the case of the litter layer of the Soil Surface treatment (slope = 0.0123; y intercept = -0.2133; coefficient of determination = 63.5%; P = 0.0058), but not for any of the other treatment-layers.

It is not clear, however, that this statistically significant relationship represents an actual relationship which should be corrected for in subsequent analyses of the data. Using corrected values (y intercept + regression residual) rather than the actual data values for the SS litter layer treatment will [[almost inevitably]] produce statistically significant differences between treatments in %P-lost, therefore this step should be taken with extreme caution.

Taking this regression relationship seriously requires that a mechanism exists to explain it. There does not seem to be much empirical or logical room for such a mechanism, though. The regression relationship

implies that P is moving out of the litter layer while the samples are waiting to be processed. There are several possible explanations for this P loss:

1) increasing mass loss (with accompanying P loss) out the bottom of the container during processing (due, presumably, to increasing dryness of the sample material);
2) translocation of P to the mulch layer above the litter layer; and
3) exit of litter-decomposer biota out the bottom of the container.

1) Increasing mass loss:

The mass loss explanation is rejected because the data show no trend of increasing mass loss with sample processing delay. In fact, there may be a slight *negative* relationship: %-mass lost seems to decrease slightly with time.

2) Translocation to mulch layer:

The translocation-to-mulch-layer explanation is rejected because there is no statistically significant increase of P in the corresponding mulch layer over time (specifically, there is no *decrease* in its %P lost over time). In fact, there is a small though not-statistically-significant *decrease* of P (increase in %P lost) in the mulch over time (P=0.086).

Of course, this rejection is invalidated if P is simultaneously moving (a) from the litter to the mulch and (b) out of the mulch into the surrounding environment, presumably in the form of motile decomposer biota. In order to assess this possibility, we estimated the mass of decomposer biota represented by the time-lag-related P loss (difference between the y-intercept and final data point). To do this, we used the values reported in Swift *et al.* (1979, p. 35) for the C/P (51) and %C (46%) of "typical Insecta" (values attributed to Allen *et al.* 1974), and we used the average initial absolute P content of the SS litter layer to calculate an average absolute time-lag-related P loss (12.9 mg). Using these values, we determined that the dry mass of such hypothetical escaping decomposers would have to be approximately 1.43 g. This turns out to be more than the average *total* mass lost from the SS mulch layer, and since there is no time-related change in mass loss from the SS mulch layer, we feel safe rejecting the possibility that there is simultaneous translocation to and decomposer escape from the mulch layer.

3) Decomposer escape from the litter layer:

The hypothetical decomposer mass values calculated above were greater than the values for actual mass loss from the SS litter layer, and since there was no time-lag-related increase in mass loss, we feel

safe rejecting the possibility that P loss occurred in the form of escaping decomposers.

Examining the data for all the treatments (Figure App.1), we see that the later data points appear to overlap each other, while the earliest two points for the SS treatment are isolated from the other data points. When those points are removed, the statistical significance of the regression disappears (P=0.108), and the r^2 drops to 37.3%. Therefore, it is conceivable that, rather than there having been a consistent time-lag effect on the %P loss in the SS-litter-layer replicates, there was, instead, a "first two samples" effect: If the first two samples were processed differently from all the others because the protocol was still new at that point, this could have had a disproportionate effect on the regression statistics.

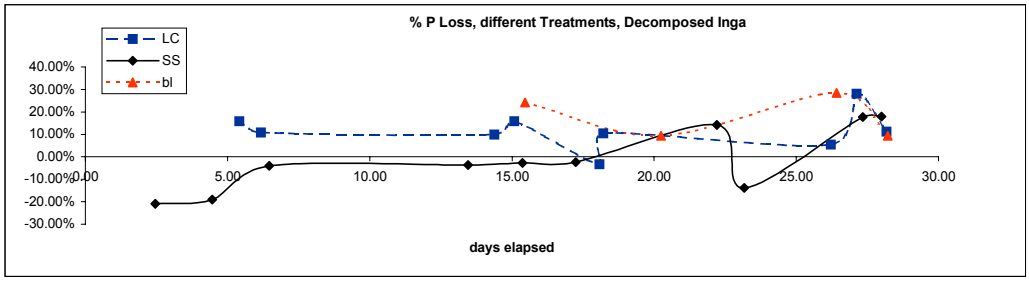

Figure App.1. %P Loss in mulch layer of all treatments vs. time of sample processing