Leaf litter nutrient uptake in an intermittent blackwater river: Influence of tree species and associated biotic and abiotic drivers

A.S. Mehring, Kevin A. Kuehn, Aaron Thompson, Catherine M. Pringle, Amy D. Rosemond, Matthew R. First, R. Richard Lowrance and George Vellidis

Rivers carry nutrients from the land to the oceans and, in doing so, are an important part of global nutrient cycles. As leaves decompose in rivers, they sequester nitrogen (N) and phosphorus (P) that might otherwise be transported downstream. This uptake of nutrients (immobilization) had long been attributed to uptake by microbial decomposers (fungi and bacteria) that colonize and decompose leaves. However, later research showed that microbial biomass in decaying plant litter could only account for a fraction of the total nutrients present, suggesting that additional mechanisms may also be important. One alternative mechanism may be the accumulation of inorganic matter on decaying litter surfaces, which contains charged particles that can bind N and P.

We compared relative contributions of microbial decomposers (biotic) and inorganic matter (abiotic) to nutrient immobilization in decaying leaf litter. We found that P immobilization in leaf litter could not be accounted for by nutrients contained in microbial biomass alone, suggesting that inorganic matter on leaf litter surfaces may play an important abiotic role in P immobilization. In contrast to P, a more complex set of factors appear to influence N immobilization in leaf litter. The combination of nutrients contained in microbial biomass and those bound to inorganic matter could not fully account for the amount of N that was immobilized. A likely source of additional N immobilization is via microbially-mediated processes, particularly the production of N-containing exoenzymes.

Leaf litter from the current (left) and previous year (right) on the bottom of an intermittent stream bed in Georgia’s coastal plain. While maple, tupelo, and oak litter can be seen in freshly-fallen litter, only oak litter can be readily distinguished among the older litter.

(enzymes excreted outside cells), which can bind with lignocellulose in decaying leaf litter to produce stable N-containing compounds. As a leaf decays, these resistant compounds can remain. Litter with higher concentrations of lignin, such as oak, immobilizes larger amounts of N and P after long periods of decomposition. This supports the idea that lignin, both by slowing mass loss and stabilizing N, may play a role in nutrient immobilization in decaying litter.

Our study shows that nutrient immobilization by decaying leaf litter may be strongly affected by microbial processes (biotic) and inorganic sediment accumulation (abiotic). Our research findings underscore that these processes are essential to understanding detrital nutrient cycling in aquatic ecosystems.
Figure S1. Leaf surfaces of freshly-shed (A) trident red maple, (B) Ogeechee tupelo, and (C) water oak leaves at 500× magnification.
Table S1.

Inorganic constituents of leaf litter (top), and Tifton soils of the Georgia coastal plain (bottom).

Mean leaf litter (combined maple, tupelo, and oak) inorganic constituents are expressed as percent of total inorganic matter adhering to litter (range in parentheses, n = 3). Inorganic constituents from Tifton soils of the Georgia coastal plain are expressed as percent total soil dry weight (Schumacher 1982). No information is available for surface horizons, but the stream is heavily incised and mineral composition of the banks is well represented by the B horizons.

Little River, leaf litter inorganic matter

<table>
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<tr>
<th>days</th>
<th>Mg</th>
<th>K</th>
<th>Ca</th>
<th>Si</th>
<th>Al</th>
<th>Fe</th>
<th>Mn</th>
<th>Na</th>
<th>clay</th>
<th>silt</th>
<th>sand</th>
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<td>2.73</td>
<td>26.35</td>
<td>7.30</td>
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<td>1.63</td>
<td>0.65</td>
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<td>3.31</td>
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<tr>
<td></td>
<td>(1.23)</td>
<td>(0.98)</td>
<td>(8.28)</td>
<td>(5.32)</td>
<td>(1.07)</td>
<td>(2.89)</td>
<td>(1.61)</td>
<td>(1.93)</td>
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<tr>
<td>36</td>
<td>1.63</td>
<td>1.07</td>
<td>12.73</td>
<td>11.72</td>
<td>4.09</td>
<td>6.98</td>
<td>2.01</td>
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<td>6.98</td>
<td>4.32</td>
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<td>(0.53)</td>
<td>(3.60)</td>
<td>(13.65)</td>
<td>(3.34)</td>
<td>(5.87)</td>
<td>(3.50)</td>
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<td>(0.64)</td>
<td>(3.08)</td>
<td>(9.89)</td>
<td>(3.98)</td>
<td>(7.32)</td>
<td>(3.17)</td>
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<td>431</td>
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Tifton soil

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<th>Fe</th>
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<th>Na</th>
<th>clay</th>
<th>silt</th>
<th>sand</th>
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<tr>
<td>Upper B (81-107)</td>
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<td>4.80</td>
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<td>5.4</td>
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<tr>
<td>Lower B (132-152)</td>
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<td>3.20</td>
<td>21.1</td>
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<td>4.39</td>
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<td>2.4</td>
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<tr>
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<td>2.60</td>
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<td>9.9</td>
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<td>54.1</td>
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<tr>
<td>C (&gt;183)</td>
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<td>2.83</td>
<td>2.00</td>
<td>23.2</td>
<td>9.8</td>
<td>8.1</td>
<td>2.81</td>
<td>56.2</td>
<td>4.5</td>
<td>39.3</td>
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Appendix 1.

Calculations and literature sources used in the development of predicted detrital nutrient models.

In calculations, all values are randomly selected from a list if literature values, and medians are generated from the results of 10,000 separate iterations of the model.

\[
N_{\text{estimated}}: \quad N_{\text{bacterial}} + N_{\text{fungal}} + N_{\text{"free" glucosamine}} + ADF-N_{\text{litter}} + \text{non-ADF}-N_{\text{litter}}
\]

\[
P_{\text{estimated}}: \quad P_{\text{bacterial}} + P_{\text{fungal}} + P_{\text{leached litter}}
\]

\[
C_{\text{fungal}}: \quad AFDM_t \times ergo_t \times \frac{1}{dm_{\text{ergo}}} \times dm_c
\]

Where:
- \(AFDM_t\) = leaf litter ash-free dry mass (g) after incubation period (t = time, in days)
- \(ergo_t\) = ergosterol content (\(\mu g\ g^{-1}\ leaf\ litter\ dry\ mass\)) after incubation period (t = time, in days)
- \(dm_{\text{ergo}}\) = ergosterol content of fungal dry mass, ranging from 2.3-11.5 \(\mu g\ ergosterol / mg\ fungal\ dry\ mass\) (Gessner & Chauvet 1993)
- \(dm_c\) = decimal fraction of C in fungal tissue, ranging from .420-.447 (Baldy & Gessner 1997; Montgomery et al. 2000)

\[
N_{\text{fungal}}: \quad \frac{C_{\text{fungal},t}}{C:N_{\text{fungal}}}
\]

Where:
- \(C_{\text{fungal},t}\) = fungal C (mg) per leaf pack after incubation period (t = time, in days)
- \(C:N_{\text{fungal}}\) = fungal C:N ratio, ranging from 6.083-16 (Newell & Statzell-Tallman 1982, Leach & Gulis 2011, personal communication,)

\[
N_{\text{"free" glucosamine}}: \quad \%_{\text{gluc free}} \times gluc_t \times AFDM_t \times 0.0782
\]

Where:
- \(\%_{\text{gluc free}}\) = \% glucosamine not in living fungal tissue = \[
\frac{d.m_{\text{gluc}} - d.m_{\text{ergo}}}{d.m_{\text{gluc}}}
\]
\( d.m.\text{gluc} \) = fungal dry mass estimated with glucosamine = \( \frac{\text{gluc}_t}{d.m.c.f.\text{gluc}} \)

\( \text{gluc}_t \) = glucosamine content (\( \mu g \) \( g^{-1} \) leaf litter dry mass) after incubation period (\( t = \) time, in days)

\( d.m.c.f.\text{gluc} \) = glucosamine to fungal dry mass conversion factor, ranging from 3.1-53.5 \( \mu g \) glucosamine per g fungal dry mass (Sharma, Fisher & Webster 1977; Graça 1990)

\( d.m.\text{ergo} \) = fungal dry mass estimated from ergosterol = \( \frac{\text{ergo}_t}{d.m.c.f.\text{ergo}} \)

\( \text{ergo}_t \) = ergosterol content (\( \mu g \) \( g^{-1} \) leaf litter dry mass) after incubation period (\( t = \) time, in days)

\( d.m.c.f.\text{ergo} \) = ergosterol to fungal dry mass conversion factor, ranging 2.3-11.5 \( \mu g \) ergosterol per mg fungal dry mass (Gessner & Chauvet 1993)

\( AFDM_t \) = leaf litter ash-free dry mass (g), after incubation period (\( t = \) time, in days)

0.0782 = percent concentration of N in glucosamine

\( N_{\text{bacterial}} \):

\[ \frac{C_{\text{bacterial},t}}{C:N_{\text{bacterial}}} \]

Where:

\( C_{\text{bacterial},t} \) = bacterial C (mg pack\(^{-1}\)) after incubation period (\( t = \) time, in days)


\( P_{\text{fungal}} \):

\[ \frac{C_{\text{fungal},t}}{C:P_{\text{fungal}}} \]

Where:

\( C_{\text{fungal},t} \) = fungal C (mg pack\(^{-1}\)) after incubation period (\( t = \) time, in days)

\( C:P_{\text{fungal}} \) = fungal C:P ratio, ranging from 40-203 (Leach & Gulis, 2011, personal communication)

\( P_{\text{bacterial}} \):
\[
\frac{C_{\text{bacterial},t}}{C: P_{\text{bacterial}}}
\]

Where:
\[C_{\text{bacterial},t}\] = bacterial C (mg pack\(^{-1}\)) after incubation period (t = time, in days)


\[N_{\text{leached litter}}\]:

\[(AFDM_{t=0} \times N_{t=0}) \times (1 - \%N_{\text{loss leaching}})\]

Where:
\[AFDM_{t=0}\] = initial (pre-incubation) leaf litter ash-free dry mass (g)

\[N_{t=0}\] = initial (pre-incubation) leaf litter N content (mg g\(^{-1}\))

\[\%N_{\text{loss leaching}}\] = percentage of total leaf litter N lost during 0-10 days of leaching, ranging 0-0.25 (Melillo et al. 1984; Ibrahima, Joffre & Gillon 1995)

\[\text{ADF-N}_{\text{litter}}\]:

\[(\%ADF_{t} \times AFDM_{t}) \times ADF\%N_{\text{litter}}\]

Where:
\[\%ADF_{t}\] = ADF( cellulose + lignin) % concentration in litter, after incubation period (t = time, in days)

\[AFDM_{t}\] = leaf litter ash-free dry mass (g), after incubation period (t = time, in days)

\[ADF\%N_{\text{litter}}\] = initial percent concentration of N in ADF (cellulose + lignin), ranging 0.006-0.0105 (Suberkropp, Godshalk & Klug 1976)

\[\text{non-ADF-N}_{\text{litter}}\]:

\[(N_{\text{leached litter}}/ AFDM_{t=0}) \times AFDM_{t} - (ADF\%N_{\text{litter}, t=0} \times ADF_{t})\]

Where:
\[AFDM_{t=0}\] = initial (pre-incubation) leaf litter ash-free dry mass (g)

\[AFDM_{t}\] = leaf litter ash-free dry mass (g), after incubation period (t = time, in days)

\[ADF_{t}\] = acid-detergent fiber (g ADF [cellulose + lignin]), after incubation period (t = time, in days)

\[P_{\text{leached litter}}\]:

\[(AFDM_{t=0} \times P_{t=0}) \times (1 - \%P_{\text{loss leaching}})\]
Where:

\[ AFDM_{t=0} = \text{initial (pre-incubation) leaf litter ash-free dry mass (g)} \]

\[ P_{t=0} = \text{initial (pre-incubation) leaf litter P content (mg g}\textsuperscript{-1}) \]

\[ \%P_{\text{loss,leaching}} = \text{percentage of total leaf litter P lost during 24 hours of leaching, ranging 0.234-0.397 (Meyer 1980; Qiu, McComb & Bell 2002)} \]

**References**


Appendix 2

Sample R code for modeled detrital N, maple litter day 36

nreps=10000 # set number of reps to 10,000


bact.c <- c(0.235285158, 0.290117652, 0.233369914) # bacterial C in mg/g, adjust values by sampling date

leafmass <- c(6.981486141, 7.361650963, 6.446215643, 6.840437937, 6.744755318) # in grams, adjust values by sampling date

randratio <- sample(bact.cnratio,nreps,replace=T) # randomly sampling literature bacterial C:N ratios

randbactc <- sample(bact.c,nreps,replace=T) # randomly sampling the observed bacterial C

randleafmass <- sample(leafmass,nreps,replace=T) # randomly sampling current leaf mass

bact.c.perpack <- (randbactc*randleafmass)*.001 # bacterial C per pack, in grams

rand.bact.c.perpack <- sample (bact.c.perpack,nreps,replace=T) # random sampling

bactn<- (randbactc/randratio)*randleafmass # bacterial N in mg per pack, flexible stoichiometry

rigidbactn<- (randbactc/6.625)*randleafmass # bacterial N in mg per pack, Redfield stoichiometry

rand.rigidbactn <- sample(rigidbactn,nreps,replace=T) # random sampling
```
rand.cnratio <- runif(nreps, 6.083, 16)  # range in fungal C:N from Newell and Statzell-Tallman 1982, D. Leach and V. Gulis personal communication estimates

ergo <- c(94.88966312, 139.136144, 143.6007342, 166.4844645, 178.4854369)  # range in fungal C:N from Newell and Statzell-Tallman 1982, D. Leach and V. Gulis personal communication estimates

derergo <- sample(ergo, nreps, replace=T)  # random sampling

glucosamine <- c(903.14, 814.60, 1057.11)  # glucosamine in ug/g AFDM, adjust by sampling date

randchitin <- sample(glucosamine, nreps, replace=T)  # random sampling

fungi.cergo.ratio <- c(2.3, 2.4, 2.6, 2.9, 3, 3.3, 3.3, 3.4, 3.5, 3.5, 3.7, 3.8, 3.9, 4, 4.2, 4.2, 4.2, 4.5, 4.5, 4.6, 4.6, 4.7, 4.7, 4.9, 5, 5, 5.1, 5.3, 5.8, 6.5, 6.8, 6.8, 7.3, 7.7, 8, 8.3, 8.5, 10, 10, 10.1, 10.2, 10.2, 11.2, 11.5)  # ug ergosterol/mg fungal dry mass, values from Gessner & Chauvet 1993

fungi.cchitin.ratio <- c(48, 40, 40, 33, 17, 16, 14.5, 13, 12, 8, 53.5, 19.6, 14.4, 10.2, 9.6, 10.6, 9.6, 13.5, 18, 44, 16.6, 11.8, 8.2, 7.7, 8.5, 7.7, 11.2, 14.6, 35.6, 18.5, 13.2, 6.2, 7.3, 5.6, 9.4, 4.5, 15.3, 26.5, 15.6, 10.7, 3.1, 5.5, 5, 7.8, 2.3, 20.2)  # ug glucosamine/mg fungal dry mass, from Manuel Graca’s dissertation and Sharma et al 1977

rand.fungi.cergo.ratio <- sample(fungi.cergo.ratio, nreps, replace=T)  # random sampling

rand.fungi.cchitin.ratio <- sample(fungi.cchitin.ratio, nreps, replace=T)  # random sampling

fungaldm <- (randergo/rand.fungi.cergo.ratio) * randleafmass  # fungal dry mass, in mg per pack, flexible conversion factor

rigidfungaldm <- (randergo/5.5) * randleafmass  # fungal dry mass, with fixed conversion factor

chitinfungaldm <- (randchitin/rand.fungi.cchitin.ratio) * randleafmass  # fungal dry mass based on glucosamine, in mg per pack

rand.fungaldm <- sample(fungaldm, nreps, replace=T)

rand.rigidfungaldm <- sample(rigidfungaldm, nreps, replace=T)

rand.chitinfungaldm <- sample(chitinfungaldm, nreps, replace=T)

defaufungus <- rand.chitinfungaldm-rand.fungaldm  # “dead fungal tissue”, flexible conversion factor

rigiddeaufungus <- rand.chitinfungaldm-rand.rigidfungaldm  # “dead fungal tissue”, fixed conversion factor

rand.deaufungus <- sample(deaufungus, nreps, replace=T)

randrigiddeaufungus <- sample(rigiddeaufungus, nreps, replace=T)

percentchitindead <- (rand.chitinfungaldm-
```

rand.fungaldm)/rand.chitinfungaldm # percentage of glucosamine not contained in living fungal tissue

rigidpercentchitinindead <- (rand.chitinfungaldm-
rand.rigidfungaldm)/rand.chitinfungaldm # percentage of glucosamine not contained in living fungal tissue, fixed ergosterol:dry mass ratio

rand.percentchitinindead <-
sample(percentchitinindead,nreps,replace=T)
rand.rigidpercentchitinindead <-
sample(rigidpercentchitinindead,nreps,replace=T)

excesschitin <- randchitin*rand.percentchitinindead # litter concentration of glucosamine not in living fungal tissue, in ug/g leaf AFDM, flexible ergosterol:dry mass c.f.

rigidexcesschitin <- randchitin*rand.rigidpercentchitinindead # litter concentration of glucosamine not in living fungal tissue, in ug/g leaf AFDM, fixed ergosterol:dry mass conversion factor

rand.excesschitin <- sample(excesschitin,nreps,replace=T)
rand.rigidexcesschitin <-
sample(rigidexcesschitin,nreps,replace=T)

excesschitinperpack <- rand.excesschitin*randleafmass # glucosamine not bound to living fungal tissue, in ug per pack

rigidexcesschitinperpack <- rand.rigidexcesschitin*randleafmass
rand.excesschitinperpack <-
sample(excesschitinperpack,nreps,replace=T)
rand.rigidexcesschitinperpack <-
sample(rigidexcesschitinperpack,nreps,replace=T)

excesschitinNperpack <- (.0782*rand.excesschitinperpack)*.001 # glucosamine N not bound in living fungal biomass, in mg N per pack

rigidexcesschitinNperpack <-
(.0782*rand.rigidexcesschitinperpack)*.001
rand.excesschitinNperpack <-
sample(excesschitinNperpack,nreps,replace=T)
rand.rigidexcesschitinNperpack <-
sample(rigidexcesschitinNperpack,nreps,replace=T)

fungal.C.ratio <- c(.438, .447, .42, .42, .426, .432, .43) # fungal percent C, values from Montgomery et al 2000 and Baldy and Gessner 1997
rand.fungal.C.ratio <- sample(fungal.C.ratio,nreps,replace=T)
fungic <- (randergo/rand.fungi.ergo.ratio) *
rand.fungal.C.ratio # calculate fungal C
rigidfungic <- (randergo/5.5)*rand.fungal.C.ratio # calculate fungal C, same conversion factor
rand.rigidfungic <- sample(rigidfungic,nreps,replace=T)
rand.fungic <- sample(fungic,nreps,replace=T)
fungin <- (rand.fungic/rand.cnratio)*randleafmass # calculate fungal N, flexible stoichiometry
rigidfungin <- (rand.rigidfungic/6.625)*randleafmass # calculate fungal N, Redfield stoichiometry
rand.rigidfungin <- sample(rigidfungin,nreps,replace=T)
fungi.c.perpack <- (rand.fungic*randleafmass)*.001 # fungal C, in grams per leaf litter pack
rigidfungicperpack <- (rand.rigidfungic*randleafmass)*.001 # fungal C, in grams per leaf litter pack
rand.fungi.c.perpack <- sample(fungi.c.perpack,nreps,replace=T)
rand.rigidfungicperpack <- sample(rigidfungicperpack,nreps,replace=T)

randfungin <- sample(fungin,nreps,replace=T)
randbactn <- sample(bactn,nreps,replace=T)
micron <- randfungin+randbactn # calculate combined microbial N
rigidmicron <- rand.rigidfungin+rand.rigidbactn
randmicron <- sample(micron,nreps,replace=T)
rand.rigidmicron <- sample(rigidmicron,nreps,replace=T)

initialAFDM <- c(8.967444658, 9.074462434, 8.732307692, 8.871613472, 8.862500053) # observed initial maple leaf litter dry mass, in grams
rand.initialAFDM <- sample(initialAFDM, nreps, replace=T)
initialleaf.n <- c(10.79451822, 9.51878338, 8.640345758) # observed initial maple leaf litter litter N content (mg/g)
rand.initialleaf.n <- sample(initialleaf.n, nreps, replace=T)
initialtotalN <- rand.initialleaf.n * rand.initialAFDM # observed initial total N, in mg per pack
rand.initialtotalN <- sample(initialtotalN, nreps, replace=T)
leach.n <- c(0, 0, 0, 0, .094, .118, .25) # % initial N lost via leaching, data from Ibrahima et al 1995 (10 days), and Melillo et al 1984
rand.leach.n <- sample(leach.n, nreps, replace=T)
initNinADF <- runif(nreps,.006,.0105) # range of values for % N in ADF (cellulose + lignin), day 0, from Suberkropp et al 1976
rand.initNinADF <- sample(initNinADF, nreps, replace=T)
currNinADF <- runif(nreps,.006,.0105) # range of values for % N in ADF, day 0, from Suberkropp et al 1976
rand.currNinADF <- sample(currNinADF, nreps, replace=T)
Nlostleachedleaf <- rand.leach.n*rand.initialtotalN # calculate N lost via leaching, in total mg from initial AFDM
rand.Nlostleachedleaf <- sample(Nlostleachedleaf, nreps, replace=T)
initialADF <- c(3.473186425, 3.067062129, 3.06819675, 3.103219471, 2.637770159) # initial observed ADF (cellulose + lignin) in grams per maple leaf litter pack
rand.initialADF <- sample(initialADF, nreps, replace=T)
currentADF <- c(3.305782557, 2.812211279, 3.076028565) # current observed ADF in grams per leaf litter pack, adjust values by sampling date
rand.currentADF <- sample(currentADF, nreps, replace=T)

initialNinADF <- (rand.initialADF * rand.initNinADF)*1000 # calculate initial total N in ADF fraction, in mg per pack
currentNinADF <- (rand.currentADF * rand.currNinADF)*1000 # calculate current total N in ADF fraction, in mg per pack
rand.currentNinADF <- sample(currentNinADF, nreps, replace=T)

initialNnotinADF <- rand.initialtotalN - rand.initialNinADF # initial leaf tissue N (minus ADF fraction), in mg per pack
rand.initialNnotinADF <- sample(initialNnotinADF, nreps, replace=T)

leachcorrectedinitialNnotinADF <- rand.initialNnotinADF - rand.Nlostleachedleaf
rand.leachcorrectedinitialNnotinADF <- sample(leachcorrectedinitialNnotinADF, nreps, replace=T)

initADFnitconc <- (rand.initialNinADF/(rand.initialADF*1000)) * 100 # calculate nitrogen in initial ADF fraction of leaf tissue, as percent
rand.initADFnitconc <- sample(initADFnitconc, nreps, replace=T)
currADFnitconc <- (rand.currentNinADF/(rand.currentADF*1000)) * 100 # calculate nitrogen in current ADF fraction of leaf tissue, as percent
rand.currADFnitconc <- sample(currADFnitconc, nreps, replace=T)

leachedmass <- c(.85, .93, .87, .865, .85, .88, .91) # % total dry mass lost during 10 days of leaching, real data from Ibrahima et al
rand.leachedmass <- sample(leachedmass, nreps, replace=T)
nonADFnitconc <- (rand.leachcorrectedinitialNnotinADF / (((rand.initialAFDM*rand.leachedmass)-rand.initialADF)*1000)) * 100 # calculate nitrogen in non-ADF fraction of leaf tissue, as percent
rand.nonADFnitconc <- sample(nonADFnitconc, nreps, replace=T)

currentAFDM <- c(6.981486141, 7.361650963, 6.446215643, 6.840437937, 6.74475318) # current leaf litter AFDM in grams per pack, adjust values by sampling date
rand.currentAFDM <- sample(currentAFDM, nreps, replace=T)
nonADFAFDM <- rand.currentAFDM-rand.currentADF
rand.nonADFAFDM <- sample(nonADFAFDM, nreps, replace=T)
AFDMminusmicrobes <- rand.nonADFAFDM-((rand.fungaldm/1000)+((rand.bact.c.perpack)*2)) # non-microbial, non-ADF fraction of litter AFDM, in g per pack
rand.AFDMminusmicrobes <- sample(AFDMminusmicrobes,nreps,replace=T)
rigidAFDMminusmicrobes <- rand.nonADFAFDM-((rand.rigidfungaldm/1000)+((rand.bact.c.perpack/1000)*2))
rand.rigidAFDMminusmicrobes <- sample(rigidAFDMminusmicrobes,nreps,replace=T)
currentnonADFnit <- (rand.nonADFnitconc*10)*rand.AFDMminusmicrobes # calculate current non-ADF leaf tissue nitrogen, in mg per pack, correcting for (subtracting) microbial mass
rand.currentnonADFnit <- sample(currentnonADFnit, nreps, replace=T)
rigidcurrentnonADFnit <- (rand.nonADFnitconc*10)*rand.rigidAFDMminusmicrobes
rand.rigidcurrentnonADFnit <- sample(rigidcurrentnonADFnit, nreps, replace=T)
currentADFnit <- (rand.currADFnitconc*10)*rand.currentADF # calculate current ADF leaf tissue nitrogen, in mg per pack
rand.currentADFnit <- sample(currentADFnit, nreps, replace=T)
new.leaf.n <- rand.currentADFnit + rand.currentnonADFnit # N in leaf tissue (ADF + non-ADF), in mg per pack
randnewleafn <- sample(new.leaf.n,nreps,replace=T)
rigidnew.leaf.n <- rand.currentADFnit + rand.rigidcurrentnonADFnit
rand.rigidnewleafn <- sample(rigidnew.leaf.n,nreps,replace=T)
currentN <- c(12.51734516, 10.79485441, 12.3725835) # observed leaf litter N, in mg/g AFDM, adjust values by sampling date
rand.currentN <- sample(currentN, nreps, replace=T)
currentNperpack <- rand.currentN*rand.currentAFDM

bioticn <- randmicron+randnewleafn+randexcesschitinNperpack # calculate modeled biotic N, flexible conversion factors, flexible stoichiometry
rigidbioticn <- rand.rigidmicron+rand.rigidnewleafn+randrigidexcesschitinNperpackage # calculate combined biotic N at fixed ergosterol conversion factors and microbial stoichiometry at Redfield ratio

prob.accountedbiotic <- function(bioest,obsmean,obssd) {
x1 <- sample(bioest,10000,replace=T)
x2 <- rnorm(10000,obsmean,obssd)
sum(ifelse(x2>x1,0,1))/10000
}
prob.accountedbiotic(bioest=bioticn, obsmean=mean(currentNperpack), obssd=sd(currentNperpack))

prob.accountedrigid <- function(bioest, obsmean, obssd) {
  x1 <- sample(bioest, 10000, replace=T)
  x2 <- rnorm(10000, obsmean, obssd)
  sum(ifelse(x2>x1,0,1))/10000
}

prob.accountedrigid(bioest=rigidbioticn, obsmean=mean(currentNperpack), obssd=sd(currentNperpack))
Sample R code for modeled detrital P, maple litter day 36

nreps=10000 # set number of reps to 10,000
bact.c <- c(0.235285158, 0.290117652, 0.233369914) # observed bacterial C, in mg/g maple litter, adjust values by sampling date
leafmass <- c(6.981486141, 7.361650963, 6.446215643, 6.840437937, 6.744755318) # current maple leaf litter AFDM, in grams, adjust values by sampling date
randratio <- sample(bact.cpratio,nreps,replace=T) # randomly sampling bacterial C:P ratios
randbactc <- sample(bact.c,nreps,replace=T) # randomly sampling the observed bacterial C
randleafmass <- sample (leafmass,nreps,replace=T) # randomly sampling current maple leaf litter mass
bact.c.perpack <- (randbactc*randleafmass)*.001 # bacterial C, in grams per maple leaf litter pack
rand.bact.c.perpack <- sample (bact.c.perpack,nreps,replace=T)
bactp <- (randbactc/randratio)*randleafmass # calculate bacterial P in mg per pack, flexible stoichiometry
rigidbactp <- (randbactc/106)*randleafmass # calculate bacterial P in mg per pack, Redfield stoichiometry
rand.rigidbactp <- sample(rigidbactp,nreps,replace=T)
```
rand.cpratio <- runif(nreps,40,203) # range in C:P from D. Leach and V. Gulis 2011, personal communication

ergo <- c(94.8966312, 139.136144, 143.600734, 166.4844645, 178.4854369) # observed ergosterol concentration in ug/g maple litter AFDM, adjust values by sampling date

randergo <- sample(ergo,nreps,replace=T) # random sampling

fungi.cergo.ratio <- c(2.3, 2.4, 2.6, 2.9, 3, 3.3, 3.3, 3.4, 3.5, 3.5, 3.7, 3.8, 3.9, 4, 4.2, 4.2, 4.2, 4.5, 4.5, 4.6, 4.6, 4.7, 4.7, 4.9, 5, 5.1, 5.3, 5.8, 6.5, 6.8, 6.8, 7.3, 7.7, 8, 8.3, 8.5, 10, 10, 10.1, 10.2, 10.2, 11.2, 11.5) # flexible ergosterol:dry mass ration, in ug/mg fungal dry mass, values from Gessner Chauvet 1993

rand.fungi.cergo.ratio <- sample(fungi.cergo.ratio,nreps,replace=T) # random sampling

fungaldm <- (randergo/rand.fungi.cergo.ratio) * randleafmass # fungal dry mass, in mg per maple leaf litter pack

rand.fungaldm <- sample(fungaldm,nreps,replace=T)

fungal.C.ratio <- c(.438, .447, .42, .42, 1.426, .432, .43) # fungal percent C, values from Montgomery et al 2000 and Baldy and Gessner 1997

rand.fungal.C.ratio <- sample(fungal.C.ratio,nreps,replace=T)

fungic <- (randergo/rand.fungi.cergo.ratio) * fungaldm # calculate fungal C, flexible conversion factor

rigidfungic <- (randergo/5.5)*fungic # calculate fungal C, with fixed conversion factor

rand.rigidfungic <- sample(rigidfungic,nreps,replace=T)

fungip <- (rand.fungic/rand.cpratio)*fungic # calculate fungal P, flexible stoichiometry

rigidfungip <- (rand.rigidfungic/106)*fungip # calculate fungal P at Redfield ratio

rand.rigidfungip <- sample(rigidfungip,nreps,replace=T)

fungi.c.perpack <- (rand.fungic*randleafmass)*.001 # in grams, flexible conversion factor

rigidfungicperpack <- (rand.rigidfungic*randleafmass)*.001 # in grams, fixed conversion factor

rand.fungi.c.perpack <- sample(fungi.c.perpack,nreps,replace=T)

rand.rigidfungicperpack <- sample(rigidfungicperpack,nreps,replace=T)

randfungip <- sample(fungip,nreps,replace=T)

randbactp <- sample(bactp,nreps,replace=T)

microp <- randfungip+randbactp # calculate combined microbial P, flexible stoichiometry

rigidmicrop <- rand.rigidfungip+rand.rigidbactp # calculate combined microbial P, Redfield stoichiometry
```
rand.microp <- sample(microp, nreps, replace=T)
rand.rigidmicrop <- sample(rigidmicrop, nreps, replace=T)

initialAFDM <- c(8.967444658, 9.074462434, 8.73207692,
                 8.871613472, 8.868250053) # initial maple leaf litter AFDM
rand.initialAFDM <- sample(initialAFDM, nreps, replace=T)

initial.leaf.p <- c(0.420011864, 0.364262409, 0.36820914) # observed initial litter N data (mg/g) from my study
rand.initial.leaf.p <- sample(initial.leaf.p, nreps, replace=T)

initial.totalP <- rand.initial.leaf.p * rand.initialAFDM # initial total P, in mg per pack
rand.initial.totalP <- sample(initial.totalP, nreps, replace=T)

leach.p <- c(.353, .397, .234, .443, .348, .307, .336, .474,
             .298, .473, .37) # % initial P lost to leaching, data from Qiu et al 2002 (24 hours) - ground litter excluded, Meyer 1980 (24 hours in lab)
rand.leach.p <- sample(leach.p, nreps, replace=T)
perc.PinNDF <- 0 # assume that the neutral detergent fiber (NDF, cellulose+hemicellulose+lignin) fraction of litter has NO phosphorus
rand.perc.PinNDF <- sample(perc.PinNDF, nreps, replace=T)

P.lost.leached.leaf <- rand.leach.p * rand.initial.totalP # calculate P lost via leaching, in total mg from initial AFDM
rand.P.lost.leached.leaf <- sample(P.lost.leached.leaf, nreps, replace=T)

initial.NDF <- c(4.11149874, 3.966104002, 3.673193015,
                 4.206373236, 3.51284909) # initial maple leaf litter lignin + cellulose + hemicellulose in grams per pack
rand.initial.NDF <- sample(initial.NDF, nreps, replace=T)

initial.PinNDF <- rand.initial.totalP * rand.perc.PinNDF # calculate total P in NDF fraction, in mg per pack
rand.initial.PinNDF <- sample(initial.PinNDF, nreps, replace=T)

initial.P.not.in.NDF <- rand.initial.totalP - rand.initial.PinNDF # initial leaf tissue P (minus NDF fraction), in mg per pack (always subtracting 0)
rand.initial.P.not.in.NDF <- sample(initial.P.not.in.NDF, nreps, replace=T)

leach.corrected.initial.P.not.in.NDF <- rand.initial.P.not.in.NDF - rand.P.lost.leached.leaf
rand.leach.corrected.initial.P.not.in.NDF <- sample(leach.corrected.initial.P.not.in.NDF, nreps, replace=T)

NDF.phos.conc <- (rand.initial.PinNDF/(rand.initial.NDF*1000)) * 100 # calculate phosphorus in NDF fraction of leaf tissue, as percent (zero)
rand.NDF.phos.conc <- sample(NDF.phos.conc, nreps, replace=T)

leached.mass <- c(.85, .93, .87, .865, .85, .88, .91) # % total
leaf litter dry mass remaining after 10 days of leaching, real data from Ibrahima et al 1995, excluding evergreen shrubs and pines

\[ \text{rand.leachedmass} \leftarrow \text{sample} \left( \text{leachedmass}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{nonNDFphosconc} \leftarrow \frac{\left( \text{rand.leachcorrectedinitialPnotinNDF}/1000 \right)}{\left( \text{rand.initialAFDM} \times \text{rand.leachedmass} \right) - \text{rand.initialNDF} \times 100}
\]

calculate phosphorus in non-NDF fraction of leaf tissue, as percent, corrected for leaching

\[ \text{rand.nonNDFphosconc} \leftarrow \text{sample} \left( \text{nonNDFphosconc}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{currentAFDM} \leftarrow \text{c} \left( 6.981486141, 7.361650963, 6.446215643, 6.840437937, 6.744755318 \right)
\]

# maple leaf litter mass remaining, in grams AFDM, adjust values by sampling date

\[ \text{rand.currentAFDM} \leftarrow \text{sample} \left( \text{currentAFDM}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{currentNDF} \leftarrow \text{c} \left( 4.131479901, 3.479421593, 3.907344112 \right)
\]

# in grams per pack, adjust values by sampling date

\[ \text{rand.currentNDF} \leftarrow \text{sample} \left( \text{currentNDF}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{nonNDFAFDM} \leftarrow \text{rand.currentAFDM} - \text{rand.currentNDF}
\]

# non-fibrous leaf litter AFDM

\[ \text{rand.nonNDFAFDM} \leftarrow \text{sample} \left( \text{nonNDFAFDM}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{AFDMminusmicrobes} \leftarrow \frac{\left( \text{rand.fungaldm}/1000 \right) + \left( \text{rand.bact.c.perpack}/1000 \right) \times 2}{\text{rand.currentAFDM}}
\]

# non-microbial, non-NDF fraction of litter AFDM, in grams per maple leaf litter pack

\[ \text{rand.AFDMminusmicrobes} \leftarrow \text{sample} \left( \text{AFDMminusmicrobes}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{rigidfungaldm} \leftarrow \left( \text{randergo}/5.5 \right) \times \text{randleafmass}
\]

# fungal dry mass, in mg per pack, fixed conversion factor

\[ \text{rand.rigidfungaldm} \leftarrow \text{sample} \left( \text{fungaldm}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{rigidAFDMminusmicrobes} \leftarrow \frac{\left( \text{rand.rigidfungaldm}/1000 \right) + \left( \text{rand.bact.c.perpack}/1000 \right) \times 2}{\text{rand.currentAFDM}}
\]

\[ \text{rand.rigidAFDMminusmicrobes} \leftarrow \text{sample} \left( \text{rigidAFDMminusmicrobes}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{currentnonNDFphos} \leftarrow \left( \text{rand.nonNDFphosconc} \times 10 \right) \times \text{rand.AFDMminusmicrobes}
\]

# calculate current non-NDF leaf tissue phosphorus, in mg per pack

\[ \text{rand.currentnonNDFphos} \leftarrow \text{sample} \left( \text{currentnonNDFphos}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[
\text{rigidcurrentnonNDFphos} \leftarrow \left( \text{rand.nonNDFphosconc} \times 10 \right) \times \text{rand.rigidAFDMminusmicrobes}
\]

\[ \text{rand.rigidcurrentnonNDFphos} \leftarrow \text{sample} \left( \text{rigidcurrentnonNDFphos}, \text{nreps}, \text{replace}=\text{T} \right) \]

\[ \text{currentNDFphos} \leftarrow \left( \text{rand.NDFphosconc} \times 10 \right) \times \text{rand.currentNDF}
\]

# calculate current NDF leaf tissue phosphorus, in mg per pack (zero)

\[ \text{rand.currentNDFphos} \leftarrow \text{sample} \left( \text{currentNDFphos}, \text{nreps}, \text{replace}=\text{T} \right) \]
new.leaf.p <- rand.currentNDFphos + rand.currentnonNDFphos # P in leaf tissue, in mg per pack
randnewleafp <- sample(new.leaf.p,nreps,replace=T)

rigidnew.leaf.p <- rand.currentNDFphos +
rand.rigidcurrentnonNDFphos
rand.rigidnewleafp <- sample(rigidnew.leaf.p,nreps,replace=T)

currentP <- c(0.388145166, 0.322224747, 0.40098813) # in mg P/g maple leaf litter AFDM, adjust values by sampling date
rand.currentP <- sample(currentP,nreps,replace=T)
currentPperpack <- rand.currentP*rand.currentAFDM # observed current P remaining per maple leaf litter pack, in mg

bioticP <- randmicrop+randnewleafp # calculate combined biotic P, flexible stoichiometry, flexible conversion factors

rigidbioticP <- rand.rigidmicrop+rand.rigidnewleafp # calculate combined biotic P at fixed ergosterol:fungal dry mass conversion factor and redfield ratio

median(bioticP)
median(rigidbioticP)
median(currentPperpack)
median(bactP)
median(rigidbactP)
median(fungiP)
median(rigidfungiP)
median(currentnonNDFphos)

prob.accountedbiotic <- function(bioest,obsmean,obssd) {
x1 <- sample(bioest,10000,replace=T)
x2 <- rnorm(10000,obsmean,obssd)
sum(ifelse(x2>x1,0,1))/10000
}

prob.accountedrigid(bioest=rigidbioticP, obsmean=mean(currentPperpack),obssd=sd(currentPperpack))

prob.accountedrigid <- function(bioest,obsmean,obssd) {
x1 <- sample(bioest,10000,replace=T)
x2 <- rnorm(10000,obsmean,obssd)
sum(ifelse(x2>x1,0,1))/10000
}

prob.accountedrigid(bioest=rigidbioticP, obsmean=mean(currentPperpack),obssd=sd(currentPperpack))