Testing associations between tree species and nitrate availability: Do consistent patterns exist across spatial scales?

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A B S T R A C T

In forest ecosystems there are numerous factors that influence nitrate (NO_3) availability and retention in ways that can significantly affect receiving waters. Unfortunately these factors often co-exist and interact making it difficult to establish the importance of each individually. Three reference watersheds at the Fernow Experimental Forest (FEF) provide a unique opportunity to evaluate the influence of tree species on soil NO_3 availability across spatial scales because they differ in stream-water NO_3 concentrations despite sharing the same macro-climate, geology, hydrology, dominant soils series, stand age, pollution regime, and land-use history.

To test the strength of plant/soil associations inferred from previous work in two watersheds in the FEF, we measured NO_3 availability and the composition of tree species in a third watershed that has intermediate stream-water NO_3 concentrations. We also examined plant/soil relationships in these watersheds at the scale of individual trees.

Across the spatial scales examined – from individual trees to small watersheds – there were consistent associations between tree species and soil NO_3 availability. For individual trees, species with high underlying soil NO_3 availability included *Acer saccharum* and *Lireodendron tulipifera*, whereas species with low underlying soil NO_3 availability included *Acer rubrum*, *Nyssa sylvatica*, and *Quercus prinus.* For small plots we found plant/soil relationships that were consistent with those made apparent by sampling under individual trees of *A. saccharum*, *A. rubrum*, *N. sylvatica*, and *Q. prinus*. And at the scale of entire watersheds, those with higher stream-water NO_3 had a greater importance of tree species associated with high NO_3 availability at finer spatial scales than did the watershed with low stream-water NO_3.

The spatially-robust relationships between tree species and soil NO_3 availability at the FEF suggest that well-characterized relationships between dominant tree species and soil properties should improve our understanding of how changes in the species composition of forest trees due to human activities, or natural causes, will alter rates of NO_3 production and loss.

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1. Introduction

The increased production of nitrate (NO_3) is a pivotal event during the process of nitrogen (N) saturation in terrestrial ecosystems that can, in turn, significantly affect N losses and downstream ecosystems (Aber et al., 1998; Driscoll et al., 2003). Several empirical studies suggest that the composition of tree species may make a significant contribution to the variability in NO_3 production and loss from forest ecosystems (Lovett and Rueth, 1999; Peterjohn et al., 1999; Lovett et al., 2000). In general, these studies indicate

that sugar maple trees are associated with high NO_3 availability and a lower capacity to retain NO_3 than oak and American beech trees. For example, Lovett and Rueth (1999) found a significant, and positive, correlation between N deposition and potential rates of net nitrification for soil collected from seven sites containing sugar maple, but not for soil collected from nearby sites containing American beech. From these observations they concluded that soils under the two species responded differently to increasing levels of N deposition, and that species differences should be considered in the prediction of forest response to N deposition, and in forest management practices.

Numerous factors, however, influence NO_3 availability and retention in forest ecosystems. These include climate, geology, hydrology, soils, stand age, pollution, species composition, natural

disturbances (insect defoliation, fire, frost, etc.), and land-use history (e.g., Vitousek and Reiners, 1975; Mitchell et al., 1996; Lewis and Likens, 2000; Aber et al., 1998, 2002; Eshleman et al., 1998; Goodale and Aber, 2001; Mitchell, 2001; Hong et al., 2005; Williard et al., 2005). Unfortunately, the frequent co-existence and interaction of these factors can confound attempts to firmly establish the importance of each individually.

Several small reference watersheds at the Fernow Experimental Forest (FEF) provide a unique opportunity to evaluate the importance of tree species because they have large differences in stream-water NO_3 concentrations despite sharing the same macro-climate, geology, hydrology, dominant soils series, stand age, pollution regime, and land-use history. Furthermore, none of the forests in these watersheds have experienced severe insect defoliation (M.B. Adams *personal communication*) and fire has not been recorded since the federal government purchased the FEF in 1915 (Schuler and Gillespie, 2000).

Previous work comparing two of these watersheds (WS 4 and WS 10) suggests there may be a relationship between the composition of tree species and NO_3 availability that persists across several spatial scales (Christ et al., 2002; Peterjohn et al., 1999). At the scale of an entire catchment (~14–34 ha), the watershed with the highest levels of stream-water NO_3 (WS 4) had a greater relative importance (dominance + density + frequency) of *Acer saccharum*, *Lireodendron tulipifera*, and *Prunus serotina* – species often associated with high nutrient requirements, high foliar N levels, and high soil N availability. The same watershed had a lower relative importance of *Acer rubrum*, *Nyssa sylvatica*, *Fagus grandifolia*, and *Quercus prinus* – species with low nutrient requirements and typical of moderate-to-low soil N availability (Abrams, 1998, 2007; Lovett and Rueth, 1999; Christ et al., 2002). Although we are unaware of generalizations about relative nutrient requirements of *Amelanchier arborea*, this species also had a lower relative importance in WS 4.

At a finer spatial scale within WS 4 (the watershed with high stream-water NO_3 levels), Peterjohn et al. (1999) found a region (~8.5 ha) with very low NO_3 availability and a lower relative importance of two tree species associated with high soil N availability (*A. saccharum* and *P. serotina*), and a greater importance of tree species associated with low N availability (*N. sylvatica*, *F. grandifolia*, and *Q. prinus*). This region also had greater relative importance of *A. arborea*.

Finally, at the spatial scale of small plots (~0.03 ha) within both WS 4 and WS 10, higher net nitrification rates in forest soils were strongly correlated with higher soil pH_{Ca} ($r = 0.75$), lower soil C:N ratios ($r = -0.70$), greater base saturation ($r = 0.72$), and (to a lesser extent) the relative importance of several dominant canopy tree species (Christ et al., 2002; Peterjohn, unpublished data). Specifically, there were positive correlations between potential net nitrification and the relative importance (dominance + density) of *A. saccharum* ($r = 0.66$), and *P. serotina* ($r = 0.41$), whereas negative correlations were found between net nitrification and the importance of *A. rubrum* ($r = -0.47$), *Q. prinus* ($r = -0.44$), *N. sylvatica* ($r = -0.41$), and *A. arborea* ($r = -0.39$). No correlation was apparent at this scale between net nitrification and the importance of *L. tulipifera* ($r = 0.18$), or *F. grandifolia* ($r = -0.001$).

To more rigorously test the strength of the apparent associations observed between tree species and NO_3 availability across spatial scales at the FEF, we measured NO_3 availability and the composition of tree species in a third reference watershed (WS 13) that has intermediate, but fairly high, stream-water NO_3 concentrations (~39 μM) when compared to the other two reference watersheds (~56 and ~16 μM for WS 4 and 10, respectively). We also examined whether associations between tree species and NO_3 availability are detectable at the scale of individual canopy trees (~0.001 ha) growing across all three reference watersheds.

2. Materials and methods

2.1. Study sites

This study used three forested watersheds located on the FEF near Parsons, West Virginia (39°3′15″N, 79°42′15″W). The watersheds (WS 4, 10, and 13) are <2 km apart and have the same dominant soils series, geology, climate, stand age, and land-use history (Fig. 1). None of the forests in these watersheds have experienced severe insect defoliation and no fire damage has been recorded.

Watershed 4 consists of 38.7 ha with an average slope of 16% and an east–southeasterly aspect. The vegetation is dominated by *A. saccharum*, *A. rubrum*, *Q. rubra*, and *L. tulipifera*. Watershed 10 is smaller (15.2 ha), more steeply sloping (26%), and has a southerly aspect. The vegetation on this watershed is dominated by *A. rubrum*, *Q. prinus*, *Q. rubra*, and *F. grandifolia*. Watershed 13 is the smallest of the watersheds (14.2 ha), with steep slopes (ca. 25%), and a north–northeasterly aspect. The vegetation on this watershed, as will be shown, is dominated by *A. saccharum*, *F. grandifolia*, *Q. rubra*, and *A. rubrum*.

On all three watersheds the dominant soil type is a Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept) derived from the acidic sandstone and shale of the underlying Hampshire formation (Losche and Beverage, 1967; Taylor, 1999). The depth to bedrock is <1 m. Mean monthly air temperatures range from about −2 °C in January to about 20 °C in July. Annual precipitation (±1 SD) is about 145 ± 14.8 cm and is uniformly distributed throughout the year. Streamflow and stream-water chemistry data exist for all three watersheds since 1988. From available data, the average annual streamflow (±1 SD) for WS 4, 10, and 13 are 64.0 ± 12.8, 64.2 ± 14.7 and 98.1 ± 9.0 cm, respectively (Adams et al., 1994). The average monthly stream-water NO_3 concentrations (±1 SD) from 1990 to 2000 for WS 4, 10, and 13 were about 56 ± 11.4, 16 ± 11.9 and 39 ± 12.3 μM NO_3, respectively. The vegetation on all three watersheds regenerated naturally after being heavily cut ca. 1907. The only known disturbance since that time was a 25% reduction in the volume of standing timber caused by the Chestnut blight in the 1930s and the removal of the dead chestnut trees in the 1940s (Adams et al., 1993; Schuler and Gillespie, 2000). All watersheds at the Fernow Experimental Forest have historically received relatively high inputs of inorganic N from the atmosphere. Between 1986 and 2002, we estimate that these watersheds received an average of ~10 kg N ha^{-1} yr^{-1} in total N (wet + dry) deposition (Adams et al., 2006). For comparisons between the reference catchments, we used the average monthly stream-water NO_3 concentrations as a measure of NO_3 availability for an entire watershed, and we measured tree data in small plots to calculate species-specific importance values (relative dominance + relative density + relative frequency) for each watershed.

2.2. Plot-based sampling

In the summer and fall of 2000, we characterized the vegetation and NO_3 availability in all three reference watersheds at the FEF. This was done to assess whether patterns between NO_3 availability and tree species composition found for WS 4 and 10 persist with the inclusion of data from WS 13 – a previously un-sampled reference watershed. Following the methods of Christ et al. (2002), the living vegetation with a DBH ⩾ 5 cm was characterized using eleven evenly-spaced plots (10-m radius and 100 m apart) in WS 13 and sixteen plots in both WS 4 and 10. In all plots we also buried three anion-exchange membranes (Dynambio, Madison, Wisconsin, USA) to a depth of 7 cm for one week. This was done five times during the growing season (twice in June, twice in July, and once late in the growing season) in WS 4 and WS 10, and once in WS
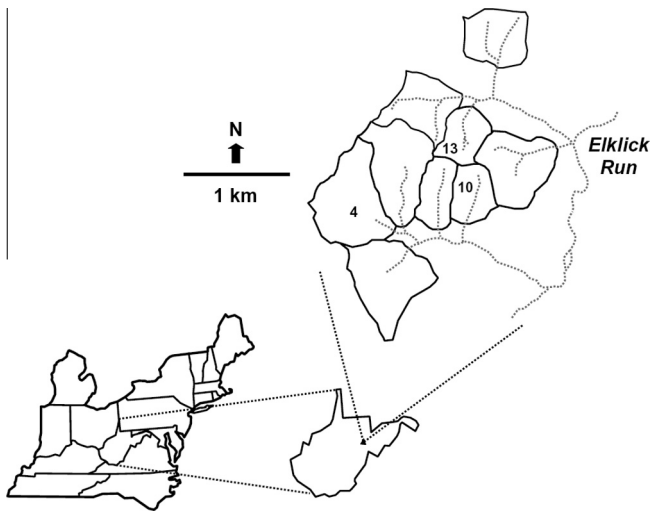
Fig. 1. Location of the Fernow Experimental Forest and the three reference watersheds used in this study (WS 4, 10, and 13).

13 (late in the growing season). After retrieval, the membranes (2×3 cm strips) were processed as described by Gress et al. (2007), and the average amount of NO_3 captured per membrane was used as a relative index of NO_3 availability in each plot. Anion exchange membranes have been used successfully in other studies (Abrams and Jarrell, 1992; McLaughlin et al., 1994; Subler et al., 1995; Cain et al., 1999), and we have found them to correlate strongly with other measures of NO_3 availability at the FEF. For example, we found a strong association ($r = 0.86$) between membrane NO_3 and the average concentration of NO_3 in soil water collected at a depth of 46 cm by tension lysimeters in WS 4 and 10 (one lysimeter/plot and an average of 18 samples/lysimeter from May 1998 through August 2000). This strong association suggests that the use of anion-exchange membranes can provide reasonable representation of NO_3 availability for the 100-m radius plots used in this study.

2.3. Tree-based sampling

To further test the strength of the association between tree species composition and NO_3 availability, we measured potential net nitrification rates and strong correlates of this process (pH and C:N ratio; Christ et al., 2002) in soils collected under fifteen trees for each of six species (Fig. 2). These species included two that have been associated with high NO_3 availability (A. saccharum and L. tulipifera), one associated with moderate-to-low NO_3 availability (F. grandifolia), and two that have been associated with low NO_3 availability (Q. prinus and N. sylvatica). We also included one species (A. rubrum) that has been associated with high NO_3 availability in some locations (Finzi et al., 1998b; Alexander and Arthur, 2010) but was associated with low NO_3 availability in plots measured at the FEF (Christ et al., 2002). Consistent with a previous study in the mountains of Appalachia (Boettcher and Kalisz, 1990), the individuals selected were canopy trees having a diameter >40 cm, and were distributed across the three reference watersheds at the FEF. In all of the watersheds we sampled under at least three individual trees for each species with the exception of N. sylvatica in WS 13 where only one individual met our size criteria.

In the summer of 2005, three soil cores (2 cm diameter) containing the upper 5 cm of mineral soil were taken from each of four quadrants around the bole of each tree, and at locations between the trunk and the vertically projected edge of the tree crown. Samples collected under each tree were combined and sieved to pass a 5.6-mm mesh. Sub-samples were then used to measure soil

pH, soil C:N ratios, and rates of potential net nitrification. Soil pH was determined by shaking 10 g (dry equivalent at 60 °C) in 100 mL of deionized water. Mixtures were allowed to stand overnight to settle and the pH of the supernatant was measured the following day using an Orion 290A pH meter. The C:N ratios of dry soils (60 °C) were measured by Dumas combustion (Bremner and Mulvaney, 1982; Nelson and Sommers, 1982) using a Carlo Erba NA 1500 N,C,S elemental analyzer. Potential net nitrification rates were calculated from the change in KCl-extractable NO_3 pools following a 30-day incubation in the dark at room temperature (Hart et al., 1994; Harlacher, 2007). Filtered extracts of the soil were analyzed for nitrate using a Lachat QuickChem AE auto-analyzer (QuickChem Methods 12-107-04-2-A and 1-B). Spike recovery tests and direct comparisons between ammonium chloride and immidazol buffer solutions were performed to ensure that Fe interference with this cadmium reduction method was not a problem (Colman et al., 2007). It was determined through regression analysis that 97% of added NO_3 spikes were recovered with the ammonium chloride buffer analysis ($R^2 = 0.995$) and that there was less than a 2% difference among results using different buffer solutions ($R^2 = 0.990$).

2.4. Statistics

Qualitative comparisons of tree species composition between the watersheds, simple correlations between the importance of individual tree species and soil NO_3 availability, and regressions between soil properties and NO_3 production rates under individual trees were made using Microsoft Excel 2010. The significance of all correlation coefficients was tested using a t-test (Sokal and Rohlf, 1981). All dependent variables for the soil samples collected under individual trees were summarized and statistically analyzed using SAS JMP software, version 5.1. A two-way ANOVA was performed to determine if the effect of the tree species on NO_3 availability in the soil depended on the watershed in which the soils were collected. A Tukey–Kramer HSD post hoc test was used in multicomparisons among species. Residual distributions were tested for normality using a Shapiro–Wilk W-test and transformed as needed to meet the assumptions of ANOVA. The data for soil pH were converted to hydronium ion concentration, cubed to achieve normality ($W = 0.974$, $p = 0.067$), and back-transformed to pH for presentation. The data for soil C:N measurements was power transformed ($W = 0.980$, $p = 0.175$) and potential rates of net nitrification were log transformed ($W = 0.989$, $p = 0.682$) to attain normally distributed residuals.

To provide a more complete analysis of species/soil relationships, we used a statistical program (Statistica, version 12) to preform two principle components analyses (PCA) based on data for the 11 tree species that had a relative importance (dominance + density + frequency) $\geqslant 5\%$ in WS 4, 10, or 13. An initial canonical correspondence analysis found the lengths of the ordination axes were less than 2 standard deviations, which indicates that the responses were monotonic (rather than unimodal), and that PCA would be appropriate (Jongman et al., 1987). The active variables used in each PCA (those used to compute factor scores) were the relative importance (dominance + density) of the tree species in each plot. For each PCA we also included soil variables for each plot that served as supplementary variables. The supplementary variables were not used in computing factor scores but were subsequently mapped onto the vector subspace in order to help identify associations between the tree species and soil variables, and associations between the soil variables themselves (StatSoft, Inc., 2014). In the first PCA we used only data from WS 4 and 10. This allowed us to include several supplementary soil variables that had been previously measured only in these watersheds (Christ et al., 2002). These soil variables included NO_3 captured
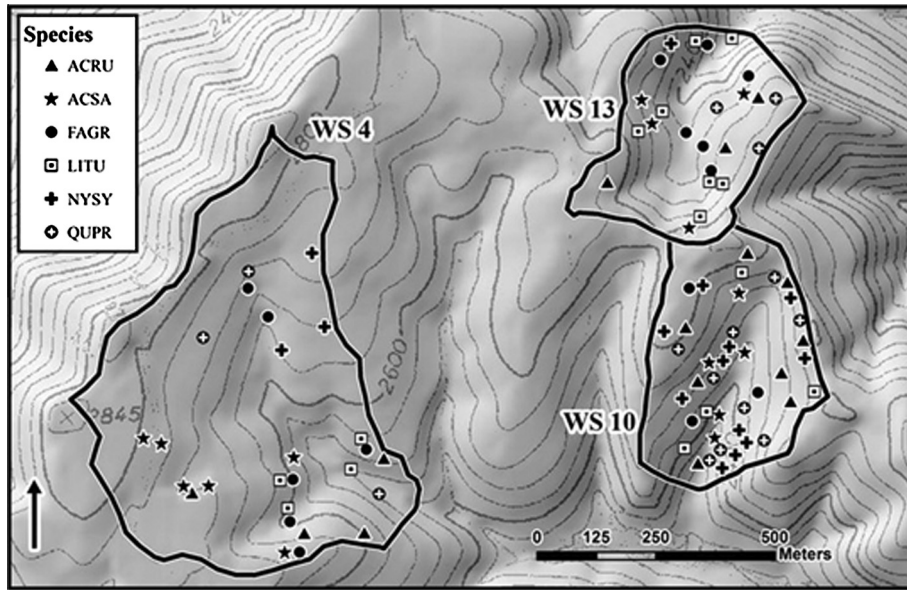
Fig. 2. Location of trees where soils were sampled in the three reference watersheds at the FEF. Species codes are: ACRU = *Acer rubrum*, ACSA = *Acer saccharum*, FAGR = *Fagus grandifolia*, LITU = *Liriodendron tulipifera*, NYSY = *Nyssa sylvatica*, and QUPR = *Quercus prinus*.

on anion-exchange membranes, lysimeter NO_3 concentrations (see Section 2.2 for more details), elevation, potential net nitrification rates, soil pH measured in distilled water, soil C:N ratios, NH_4Cl exchangeable Ca, and KCl exchangeable Al. In the second PCA we used tree data from all the reference watersheds (WS 4, 10 and 13) as the active variables, and elevation and NO_3 captured on anion-exchange membranes (the only variables measured in all plots) were included as supplementary variables. The goal of the second PCA was to determine if the patterns found in WS 4 and 10 would persist once data from WS 13 were added.

3. Results

3.1. Catchment scale

At the spatial scale of a small catchment (14–39 ha), the importance of many tree species is substantially different among the three reference watersheds (Fig. 3). Species previously associated with high NO_3 availability at the FEF (*A. saccharum*, *L. tulipifera*, and *P. serotina*) collectively account for a relative importance of ~30% in both watersheds that have high NO_3 levels (WS 4 and 13), whereas their relative importance is only 8% in the watershed with low stream-water NO_3 levels (WS 10). In contrast, species previously associated with lower NO_3 availability at the FEF (*A. rubrum*, *N. sylvatica*, *F. grandifolia*, *Q. prinus*, and *A. arborea*) collectively account for a relative importance of 25% in WS 4, 34% in WS13, and 55% in WS 10. The two watersheds with the highest stream-NO_3 concentrations (WS 4 and 13) had similar relative importance values for the tree species associated both with high and low NO_3 availability. One exception, however, was the relative importance of *F. grandifolia* which was much higher in WS 13 (15%) than WS 4 (5%). The remaining tree species in all watersheds either showed little difference in their importance between watersheds (e.g., *Q. rubra*), or had low importance and no previous evidence of being associated with NO_3 availability at the FEF.

3.2. Plot scale

At the spatial scale of small plots (~0.03 ha), new data from WS 13 were consistent with relationships between NO_3 availability
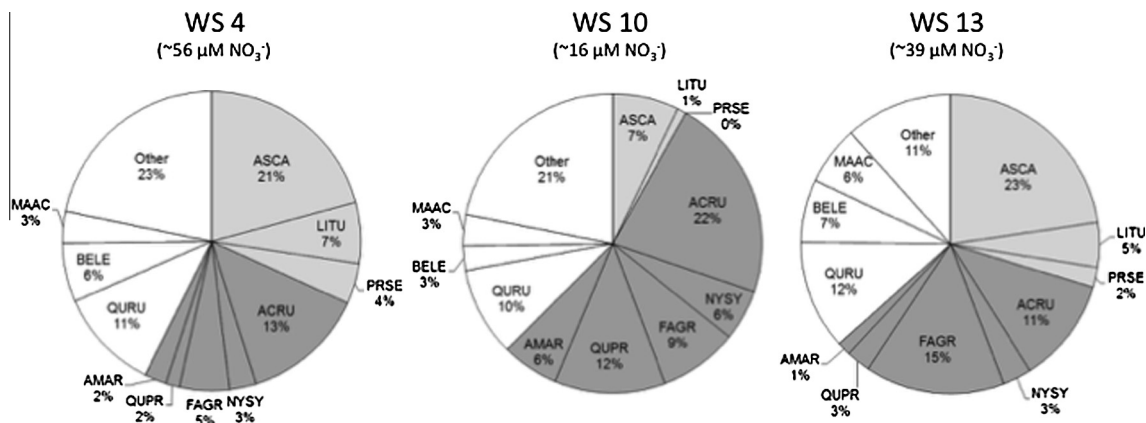

Fig. 3. Composition of tree species with a relative importance (dominance + density + frequency) ⩾5% in WS 4, 10, or 13 at the FEF. Average stream-water nitrate concentrations for each watershed in parentheses. Species codes are: ACSA = *Acer saccharum*, LITU = *Liriodendron tulipifera*, PRSE = *Prunus serotina*, ACRU = *Acer rubrum*, NYSY = *Nyssa sylvatica*, FAGR = *Fagus grandifolia*, QUPR = *Quercus prinus*, AMAR = *Amelanchier arborea*, QURU = *Quercus rubra*, BELE = *Betula lenta*, and MAAC = *Magnolia acuminata*. Light shading is for species associated with locations of higher N availability at FEF. Darker shading is for species associated with locations of lower N availability at FEF. No shading is for species whose association with N availability at the FEF is uncertain.
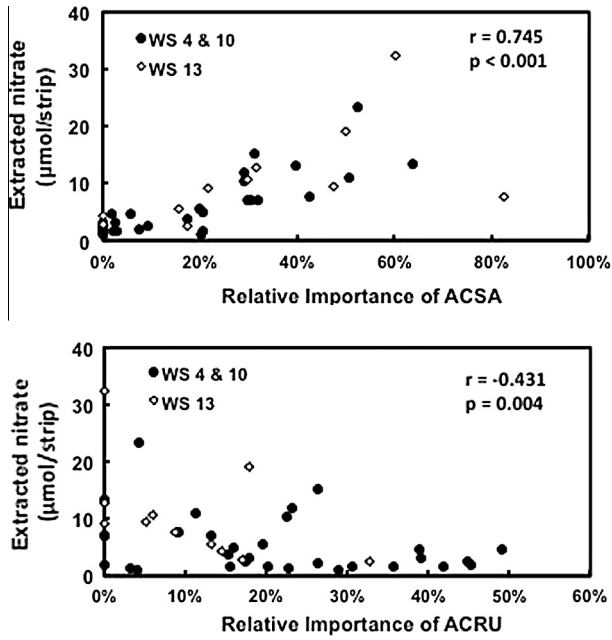
Fig. 4. The strongest positive and negative correlations found in small plots between NO$_3$ availability (assessed with anion resin membranes) and the relative importance (dominance + density) of individual tree species. Species codes are: ACSA = *Acer saccharum*, and ACRU = *Acer rubrum*.

and tree species that exist for the other watersheds (Fig. 4). Using the plots from all three watersheds, several significant correlations were found between the relative importance of various tree species and NO$_3$ availability as assessed by anion-exchange membranes. The strongest positive association was between NO$_3$ availability and the relative importance of *A. saccharum* ($r = 0.745$; $p < 0.001$) and the strongest negative association was between NO$_3$ availability and the relative importance of *A. rubrum* ($r = -0.431$; $p = 0.004$). A weaker positive association was found between NO$_3$ availability and the relative importance *P. serotina* ($r = 0.519$; $p < 0.001$), and weaker negative associations were found for *N. sylvatica* ($r = -0.421$; $p = 0.005$), *A. arborea* ($r = -0.416$; $p = 0.006$), and *Q. prinus* ($r = -0.402$; $p = 0.008$). The weaker associations also had more plots (58–81%) where the species of interest was absent, however, the direction of the associations did not change if the plots missing the species of interest were not included. At this spatial scale no significant relationships were found for any other species examined, including *L. tulipifera* and *F. grandifolia*.

The new data from small plots in WS 13 were also consistent with the relationships between tree species found in the other reference watersheds, and they did not alter the relationships between these species and NO$_3$ availability found in the other watersheds (Fig. 5).

The PCA using data only from WS 4 and 10 found that *A. saccharum* and *P. serotina* clustered together and were positively associated with high values for several measures of NO$_3$ availability (including anion resin membranes), soil pH, and exchangeable Ca (Fig. 5). In contrast, four species (*A. rubrum*, *Q. prinus*, *A. arborea*, and *N. sylvatica*) were clustered together and positively associated with low NO$_3$ availability, and high levels of exchangeable Al and high soil C:N ratios. The remaining species examined (*M. acuminata*, *F. grandifolia*, *B. lenta*, and *L. tulipifera*) were not aligned with the NO$_3$ availability gradient. The patterns observed for WS 4 and 10 were also not significantly altered by the inclusion of the data from plots in WS 13 (Fig. 5a vs. b).
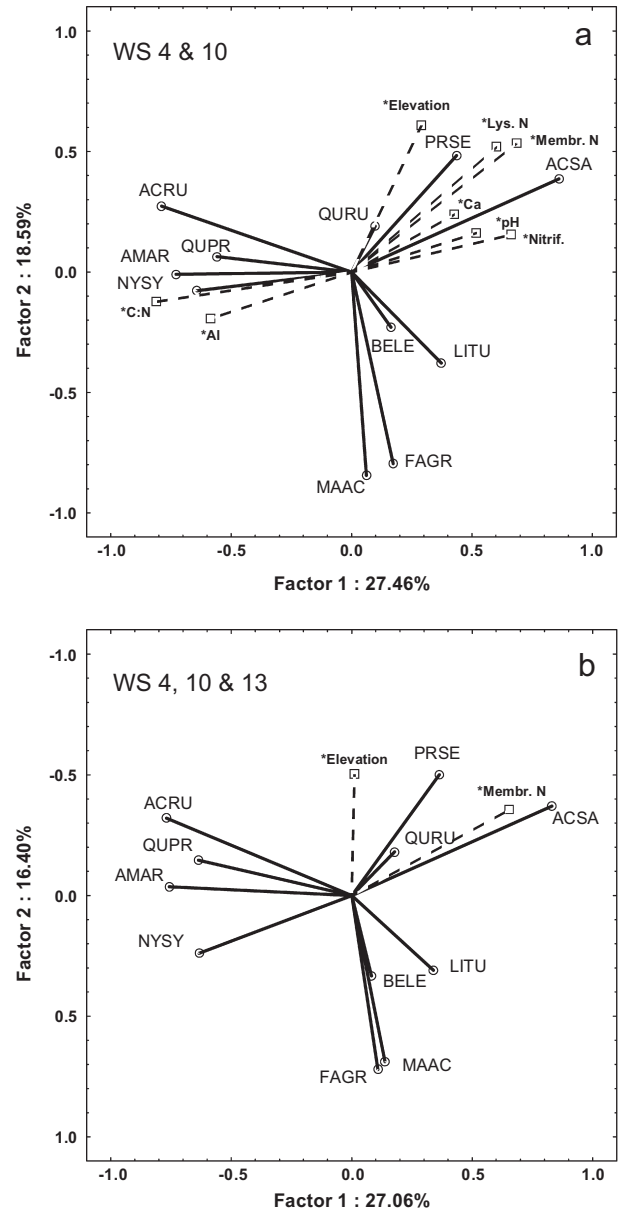

Fig. 5. Principal component analysis of tree species with a relative importance (dominance + density + frequency) $\geqslant 5\%$ in WS 4, 10, or 13 at the FEF. Supplementary variables projected onto the factor space are: Elevation; Membr. N = NO$_3$ captured by anion resin membranes; Lys. N = lysimeter NO$_3$ concentrations; Nitrif. = potential net nitrification; pH = soil pH in water; Ca = exchangeable Ca; Al = exchangeable Al; and C:N = soil carbon-to-nitrogen ratio. Species codes are the same as those used in Fig. 3.

3.3. Individual trees

At the spatial scale of soils found under the canopy of individual trees (~0.001 ha), there were many differences in soil properties and net nitrification rates between the species examined (Fig. 6). The pH of soils collected beneath canopy trees varied among species ($p < 0.001$) but did not differ between the watersheds ($p = 0.239$). Nor did the response of pH to species depend on the watershed in which the species were growing (i.e. there was no significant interaction; $p = 0.930$). Overall, the average soil pH decreased in the following order: *L. tulipifera* (3.66) > *A. saccharum* (3.61) > *F. grandifolia* (3.43) > *N. sylvatica* (3.39) > *A. rubrum* (3.22) > *Q. prinus* (3.11). Despite all soils being strongly acidic, the pH
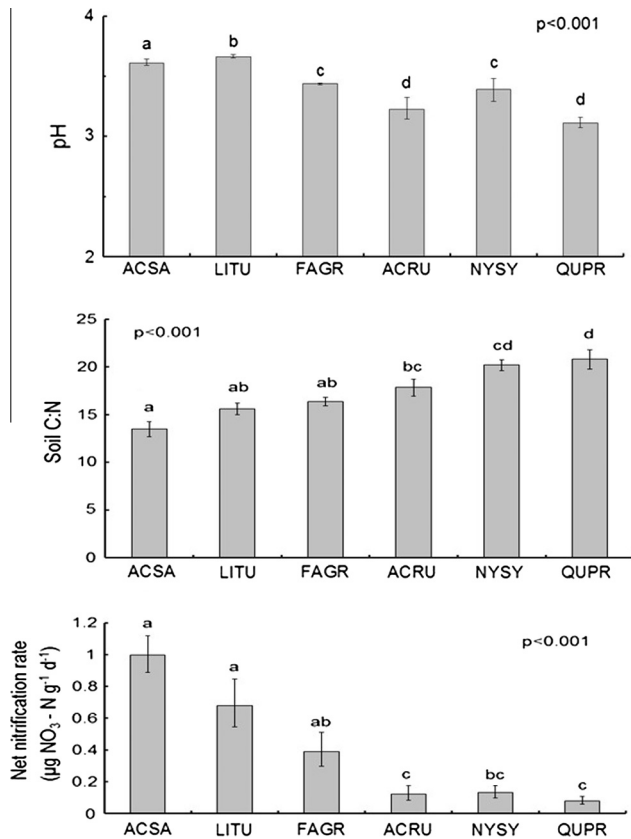
Fig. 6. pH, C:N ratios, and net nitrification rates for soils collected beneath canopy trees of different species. Bars represent mean ± 1 SE. Species with different letters denote significant differences as determined by Tukey–Kramer HSD testing. P-values for the main effect of species determined by 2-way ANOVA. Species codes are: ACSA = *Acer saccharum*, LITU = *Liriodendron tulipifera*, FAGR = *Fagus grandifolia*, ACRU = *Acer rubrum*, NYSY = *Nyssa sylvatica*, and QUPR = *Quercus prinus*.

for *L. tulipifera* and *A. saccharum* were significantly higher than all other species tested. *F. grandifolia* and *N. sylvatica* were statistically similar to one another, but different from the other species. *A. rubrum* and *Q. prinus* were also statistically similar to one another, but lower than all other species tested.

The C:N ratio of soil beneath dominant canopy trees varied among species ($p < 0.001$) but not between watersheds ($p = 0.575$). However, a significant interaction was detected ($p = 0.012$) because for one species (*Q. prinus*) the soil C:N ratio depended on the watershed in which the trees grew, with the ratio being higher in WS 13 than WS 4. When averaged across watersheds, the mean soil C:N ratio decreased in the following order: *Q. prinus* (20.81) > *N. sylvatica* (20.20) > *A. rubrum* (17.86) > *F. grandifolia* (16.37) > *L. tulipifera* (15.60) > *A. saccharum* (13.51). At the extremes, the average soil C:N ratio beneath *A. saccharum* was significantly lower than the other species except for *L. tulipifera* and *F. grandifolia*, while the average soil C:N ratio beneath *N. sylvatica* and *Q. prinus* were significantly higher than all other species except for *A. rubrum*.

Average rates of potential net nitrification from soils collected beneath dominant canopy trees of different species varied by more than an order of magnitude ($p < 0.001$). The average rates did not vary among watersheds ($p = 0.143$), nor did the response to species differ among watersheds (i.e. no significant interaction; $p = 0.195$). Mean values for net nitrification decreased in the following order: *A. saccharum* (1.00 µg NO_3-N g^{-1} d^{-1}) > *L. tulipifera* (0.68 µg NO_3-N g^{-1} d^{-1}) > *F. grandifolia* (0.39 µg NO_3-N g^{-1} d^{-1}) > *N. sylvatica* (0.13 µg NO_3-N g^{-1} d^{-1}) > *A. rubrum* (0.12 µg NO_3-N g^{-1} d^{-1}) > *Q. prinus* (0.08 µg NO_3-N g^{-1} d^{-1}). The rates measured

in soils collected beneath *A. saccharum* and *L. tulipifera* were higher than all other species sampled except *F. grandifolia*, and the average rate for soils under *A. rubrum* and *Q. prinus* were lower than all other species except *N. sylvatica*.

Across the species examined there exists a strong, negative, exponential relationship ($R^2 = 0.895$) between estimates of net nitrification rates and soil C:N ratios (Fig. 7), and a strong, positive, relationship ($R^2 = 0.751$) between net nitrification rates and soil pH (Fig. 7). The exponential relationship between net nitrification rates and soil C:N ratios is consistent with the findings of other studies in forest ecosystems (e.g. Goodale and Aber, 2001; Aber et al., 2003; Ross et al., 2004, 2009). Across a wide range of soil pH, rates of nitrification have been found to increase with increases in the soil pH of both agricultural and forest soils (Robertson, 1982; Aber and Melillo, 2001), however, given the low values and narrow range of soil pH that we measured, it is uncertain whether the apparent sensitivity to pH is a direct effect, or a response to covariates such as higher soil Ca and lower soil Al levels (Fig. 5).

4. Discussion

Across the spatial scales examined in this study – from individual trees to small watersheds – there were fairly consistent patterns found in the associations between tree species and soil NO_3 availability. At the spatial scale of individual trees (~0.001 ha), the species sampled at the FEF appear to group into three categories: (1) Species associated with high soil NO_3 availability, lower soil C:N ratios, and higher soil pH values (e.g. *A. saccharum* and *L. tulipifera*); (2) Species with intermediate – but not always statistically distinct – levels of NO_3 availability, soil C:N ratios, and soil pH (e.g. *F. grandifolia*); and (3) Tree species associated with low soil
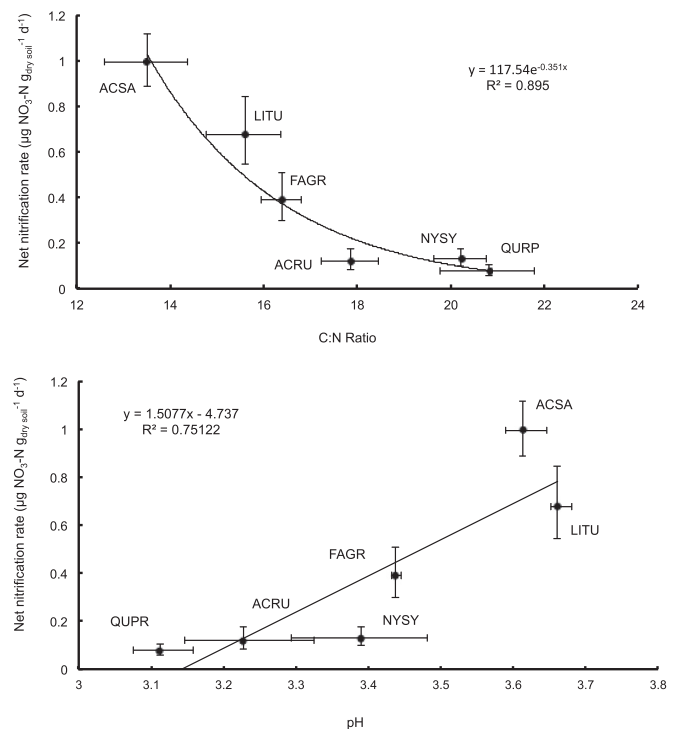

Fig. 7. The relationship, across species, between net nitrification rates under individual trees and the soil C:N ratio and pH. Values are arithmetic means for 15 trees of each species; error bars represent back transformed values for 1 SE. Regression line fit through mean values. Species codes are: ACSA = *Acer saccharum*, LITU = *Liriodendron tulipifera*, FAGR = *Fagus grandifolia*, ACRU = *Acer rubrum*, NYSY = *Nyssa sylvatica*, and QUPR = *Quercus prinus*.

NO_3 availability, higher soil C:N ratios, and lower or intermediate soil pH (e.g. *A. rubrum*, *N. sylvatica*, and *Q. prinus*). These results are similar to those found at other locations in the deciduous forest of the Eastern United States (Boettcher and Kalisz, 1990; Finzi et al., 1998a,b), and confirm that distinct differences in the properties of soils collected under individual trees of different species can persist despite being located in steep terrain that can cause the downslope transport and mixing of leaf litter (Orndorff and Lang, 1981), and despite the mixed composition of the forest which results in different species being in close proximity to each other.

At coarser spatial scales (plots, regions within a watershed, and entire watersheds) the associations between tree species and soil NO_3 availability show similar quantitative and qualitative patterns to those that were found by sampling under individual trees, suggesting that the effects of individual trees may be additive across the spatial scales we examined. Sampling at coarser spatial scales may also help to identify how other species (those not sampled individually) might be categorized with respect to their relationship with soil N availability.

At the scale of small plots ($\sim$0.03 ha) located across three reference watersheds at the FEF, patterns consistent with the results from sampling under individual trees were found for *A. saccharum*, *A. rubrum*, *N. sylvatica*, and *Q. prinus* (Figs. 4 and 5). A particularly strong positive relationship ($r = 0.745$) was found between NO_3 availability (indexed using anion-exchange membranes) and the relative importance of *A. saccharum*, and a significant negative relationship ($r = -0.431$) was found between NO_3 availability and the relative importance of *A. rubrum* (Fig. 4). And for these relationships between a species' importance and soil NO_3 availability, the data from WS 13 were consistent with the patterns found for the other reference watersheds at the FEF that have been previously studied (WS 4 and 10; Christ et al., 2002). The results from both simple correlations and a multivariate analysis (PCA) of data from the small plots also revealed a significant positive association between NO_3 availability and the relative importance of *P. serotina*, and a negative association with the importance of *A. arborea*. This suggests that if soils were sampled from under individual trees of these species, those collected under *P. serotina* would be similar to soils under *A. saccharum*, and soils collected under *A. arborea* would be similar to those found under *A. rubrum*, *N. sylvatica*, and *Q. prinus*. It is surprising that, given the results from the soils collected under individual trees, there was not a significant correlation in small plots between NO_3 availability and the relative importance of *F. grandifolia* and *L. tulipifera*, and this may indicate that any relationship at this spatial scale (if it exists) was masked by the stronger effects of other factors (e.g. elevation; Fig. 5) or by species that are particularly dominant in plots that lack *F. grandifolia* or *L. tulipifera*.

At essentially the same spatial scale (0.04-ha plots), Gilliam et al. (2001) used a multivariate approach and did not find evidence for an association between tree species and soil NO_3 availability in WS 4 at the FEF. However, their results were based on data from 15 plots in one of the reference watersheds, and only 5 of the plots were associated with low levels of NO_3 availability. As a consequence we feel that their results underscore the need for the approach taken in this study where the association between tree species and NO_3 availability was examined using more samples (43 plots) and in all of the reference watersheds at the FEF.

At the coarser spatial scale of a small region ($\sim$8 ha) within WS 4, previous research at the FEF found qualitative associations between soil NO_3 availability and tree species that are consistent with patterns found in this study for individual trees and for small plots (Peterjohn et al., 1999). In a south-facing region of WS 4 we found low NO_3 availability and a much greater relative importance of *N. sylvatica*, *Q. prinus*, *A. arborea*, and *F. grandifolia* than what is found in the majority ($\sim$30 ha) of WS 4 which had much higher

levels of NO_3 availability. In addition, the same region had a much lower relative importance of *A. saccharum* and *P. serotina* compared to the larger, NO_3-rich, portion of the watershed. No large differences in the relative importance of either *A. rubrum* or *L. tulipifera* were apparent. Although the link between NO_3 availability and tree species at this spatial scale was qualitative, we hypothesized that the composition of tree species might account for the variable degrees of NO_3 availability that is often found in forests that receive similar levels of elevated N deposition (Peterjohn et al., 1999).

Finally, at the scale of entire watersheds at the FEF ($\sim$14–34 ha), those with higher stream-water NO_3 levels (WS 4 and 13) had a greater collective importance of tree species that are associated with high NO_3 availability at finer spatial scales than did the watershed with low stream-water NO_3 (WS 10; Fig. 3). In contrast, the watershed with low stream-water NO_3 levels (WS 10) had a greater collective importance of tree species associated with low NO_3 availability than did the watersheds with elevated stream-water NO_3 levels. Although this qualitative comparison suggests that one should be able to predict average stream-water NO_3 levels from the composition of tree species found in a watershed, such predictions would likely require modeling of water flow through the variable source areas of a watershed, measuring the species composition of those areas, and then linking the species composition to the groundwater chemistry of each area (either empirically or mechanistically).

The spatially-robust relationships between tree species and soil NO_3 availability at the FEF were especially apparent for *A. saccharum*, and are consistent with studies conducted in the Catskill and Adirondack Mountains in New York (Lovett et al., 2000, 2002; Mitchell et al., 2003; Lovett et al., 2004). Collectively, the similarity of these results is striking and it supports the hypothesis that *A. saccharum* (sugar maple) may be a widespread, keystone species with respect to N cycling in the deciduous forests of the eastern U.S. (Lovett and Mitchell, 2004). And although we cannot determine whether tree species cause, or merely reflect, patterns of soil NO_3 availability, our data do support the hypothesis that the nature of leaf litter could alter soil C:N ratios in ways that influence rates of nitrification (Lovett et al., 2002; Lovett and Mitchell, 2004).

While the relationship between *A. saccharum* and high levels of NO_3 availability appears to be widespread, in some locations it may be overwhelmed by the influence of other species (Ross et al., 2009; Ross and Wemple, 2011), and differences among species in their plant/soil associations may be context dependent (Lovett and Rueth, 1999; Cross and Perakis, 2011) – even for species that are closely related taxonomically. For example, the two species of maple we examined at the FEF (*A. rubrum* and *A. saccharum*) differed dramatically in their associations with soil NO_3 availability. Specifically, the soils under *A. rubrum* had lower rates of net nitrification and higher soil C:N ratios than soils collected under *A. saccharum*. A similar result for soil C:N ratios was reported for forest soils in New York (Lovett et al., 2002) and for soil C:N ratios and soil nitrification rates in New Hampshire (Venterea et al., 2003). However, a study in Connecticut found that soil under individual trees of *A. rubrum* had high values of net nitrification, low C:N ratios, and values for both nitrification and C:N ratios that were essentially identical to those for soil collected under nearby trees of *A. saccharum* (Finzi et al., 1998b). Additionally, in a forested site in Kentucky the rates of net nitrification in soils under *A. rubrum* were significantly greater than soils under *Q. prinus* (Alexander and Arthur, 2010) whereas they were essentially the same in the FEF.

It is unclear why *A. rubrum* exhibits a variable relationship to soil NO_3 availability, but its ability to grow in a wide range of environmental conditions (Abrams, 1998) may influence the nature of

its leaf litter in site-specific ways. Differences in the nature of leaf litter could then alter soil pH and/or soil C:N ratios in ways that strongly influence rates of net nitrification. For example, at the scale of small plots in the reference watersheds at the FEF, the relative importance of *A. rubrum* was not only negatively associated with NO_3 availability (Fig. 4), but it was also negatively correlated with soil pH, and positively correlated with the soil C:N ratio in WS 4 and 10 (Fig. 5). Furthermore, when averaged over two amounts (ambient and 2× ambient), and after two years of treatment, experimental additions of senesced *A. rubrum* leaves to microcosms containing a uniform mix of mineral soil from the FEF increased the soil C:N ratio, lowered the NO_3 levels in leachate, reduced total NO_3 export, and resulted in lower leachate pH when compared to soils receiving additions of leaf litter from *A. saccharum* (Harlacher, 2007). Thus, the characteristics of *A. rubrum* leaf litter may account for at least a portion of this species' association with low, rather than high, NO_3 availability in the central Appalachian forests of the FEF. It is uncertain whether *A. rubrum* leaf litter in other locations has different characteristics (such as C:N ratios) compared to what is found at the FEF or whether the properties of its leaf litter are more spatially variable than those of *A. saccharum*, but a deeper investigation seems warranted into the location-dependent differences in plant/soil relationship for these two widespread, congeneric species.

Ostensibly our results seem consistent with studies suggesting that soil Ca may influence NO_3 availability and loss (Williard et al., 2005; Christopher et al., 2006; Page and Mitchell, 2008a, 2008b). From the results of these studies it is hypothesized that Ca-rich parent material favors Ca-demanding tree species (e.g. *A. saccharum* and *Tilia americana*) which, in turn, produce leaf litter with characteristics (e.g. low lignin:N or low C:N ratios) that promote greater rates of nitrification (Christopher et al., 2006). For the two watersheds at the FEF where numerous soil variables had been previously measured (WS 4 and 10; Christ et al., 2002), a PCA revealed a positive association between several indices of soil NO_3 availability, exchangeable Ca, and soil pH (Fig. 5a). It also revealed a negative association between NO_3 availability, soil C:N ratios, and exchangeable Al. However, the study sites at the FEF did not vary with respect to their underlying geology, which is dominated by the sandstones and shales of the Rowlesburg and Canon Hill members of the Upper Devonian Hampshire Formation (Taylor, 1999). Thus, at our sites factors other than geology (e.g. aspect) may have influenced the spatial distribution of tree species; and the associations between soil Ca, NO_3 availability, and tree species may have been driven by the species themselves rather than the underlying geology.

In the absence of potentially confounding factors (e.g. differences in land-use history, stand age, climate, and geology), the spatially robust patterns between the composition of tree species and soil NO_3 availability at the FEF suggest that NO_3 availability (and perhaps other ecosystem properties) at coarser spatial scales can strongly reflect, and may be reasonably inferred from, the species composition of dominant canopy trees. For larger areas, if well-characterized relationships between tree dominant species and soil properties can be established, and if the interactive effects of other factors (e.g. geology, climate, etc.) can be accounted for, then data from forest inventories and remotely sensed images offer the chance for improved estimates of large-scale ecosystem properties. Plant/soil relationships such as those found at the FEF also support the importance of their inclusion into ecosystem models, like the LINKAGES model (Pastor and Post, 1986, 1988; Post and Pastor, 1996), that are used to better understand how changes in the species composition of forest trees due to human activities, or natural causes, will alter rates of nitrate production and loss.

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