Supplementary Material

Table S1. Foliar Nitrate Reductase Activity (NRA, μmol $g^{-1}$ $h^{-1}$) of less common herb-layer species at the Fernow Experimental Forest, West Virginia, USA, sorted by plant type (W – woody seedling, S/V – shrub or vine, F – fern, H – herb). NRA was averaged across all available measurements (*n*), i.e., taken during three sampling campaigns and from all four watersheds in the study. Min - minimum, Max - maximum, SD - standard deviation.

| **Species** | **Common Name** | **Plant Type** | ***n*** | **Average NRA** | **Min NRA** | **Max NRA** | **SD** |
|---|---|---|---|---|---|---|---|
| *Acer rubrum* | Red maple | W | 5 | 0.108 | 0.002 | 0.234 | 0.093 |
| *Betula lenta* | Sweet birch | W | 2 | 0.066 | 0.000 | 0.132 | 0.093 |
| *Fraxinus spp.* | Ash | W | 11 | 0.108 | 0.000 | 0.347 | 0.104 |
| *Hamamelis virginiana* | Witchhazel | W | 6 | 0.025 | 0.000 | 0.047 | 0.015 |
| *Ilex montana* | Big leaf holly | W | 1 | 0.030 | | | |
| *Liridendron tulipifera* | Tulip-poplar | W | 1 | 0.030 | | | |
| *Magnolia acuminata* | Cucumber tree | W | 16 | 0.495 | 0.112 | 1.115 | 0.296 |
| *Nyssa sylvatica* | Black gum | W | 2 | 0.228 | 0.157 | 0.299 | 0.100 |
| *Prunus serotina* | Black cherry | W | 3 | 0.015 | 0.000 | 0.025 | 0.013 |
| *Quercus montana* | Chestnut oak | W | 1 | 0.040 | | | |
| *Quercus rubra* | Red oak | W | 20 | 0.083 | 0.025 | 0.187 | 0.046 |
| *Quercus velutina* | Black oak | W | 3 | 0.181 | 0.127 | 0.281 | 0.086 |
| *Sassafras albidum* | Sassafras | W | 3 | 0.068 | 0.028 | 0.128 | 0.053 |
| *Berberis thunbergii* | Japanese barberry | S/V | 1 | 0.619 | | | |
| *Menziesia pilosa* | Minniebush | S/V | 3 | 0.003 | 0.000 | 0.005 | 0.003 |
| *Parthenocissus quinquefolia* | Virginia creeper | S/V | 2 | 1.000 | 0.614 | 1.387 | 0.547 |
| *Vaccinium pallidum* | Blueberry | S/V | 8 | 0.041 | 0.000 | 0.109 | 0.048 |
| *Viburnum acerifolium* | Mapleleaf viburnum | S/V | 6 | 0.085 | 0.000 | 0.183 | 0.062 |
| *Athyrium filix-femina* | Lady fern | F | 3 | 0.121 | 0.036 | 0.191 | 0.079 |
| *Osmundastrum cinnamomeum* | Cinnamon fern | F | 1 | 0.113 | | | |
| *Arisaema triphyllum* | Jack-in-the-pulpit | H | 2 | 0.446 | 0.339 | 0.553 | 0.151 |
| *Symphyotrichum/Eurybia* spp. | Aster | H | 5 | 0.047 | 0.000 | 0.130 | 0.054 |
| *Cypripedium acaule* | Lady's slipper | H | 1 | 0.248 | 0.248 | 0.248 | |
| *Dioscorea quaternata* | Wild yam | H | 3 | 0.123 | 0.056 | 0.209 | 0.078 |
| *Disporum lanuginosum* | Fairy bells | H | 8 | 0.276 | 0.020 | 0.611 | 0.166 |
| *Eurybia divaricata* | White wood aster | H | 4 | 0.050 | 0.000 | 0.130 | 0.063 |
| *Laportea canadensis* | Stinging nettle | H | 1 | 0.454 | | | |
| *Medeola virginiana* | Indian cucumber root | H | 12 | 0.313 | 0.000 | 1.279 | 0.342 |
| *Polygonum virginianum* | Virginia knotweed | H | 6 | 2.087 | 0.640 | 5.719 | 1.822 |
| *Trillium* spp. | Trillium | H | 1 | 0.100 | 0.100 | 0.100 | |
| *Uvularia perfoliata* | Bellwort | H | 1 | 0.212 | | | |

Table S2. Specific Leaf Area (SLA, $m^2 g^{-1}$) of red and sugar maple collected at the Fernow Experimental Forest, West Virginia, USA, in August 1998. Each data point is based on a single branch shot down from randomly selected canopy trees. Specific Leaf Weight (SLW) is the inverse of SLA.

| *n* | **Red maple** | **Sugar maple** |
|---|---|---|
| 1 | 186 | 116 |
| 2 | 166 | 174 |
| 3 | 88 | 184 |
| 4 | 210 | 229 |
| 5 | 171 | |
| 6 | 162 | |
| 7 | 153 | |
| 8 | 72 | |
| 9 | 152 | |
| 10 | 172 | |
| Average SLA (SLW) | 153.2 (0.0065) | 175.9 (0.0057) |

Table S3**.** Summary of statistical analyses of the effect of watershed-level treatment (WS) and species of overstory maple (M), their interaction, and other factors on NRA of plant tissues collected at the Fernow Experimental Forest, West Virginia, USA.

| Dependent Variable | Predictor | ANOVA Model Description |
|---|---|---|
| 1. Summer Overstory Maple Foliage NRA | WS<br>M<br>WSxM<br>Time | Analyses were conducted with a) watershed pairs and b) all four watersheds in same model for greater statistical power to detect a Maple effect (i.e., a difference between red and sugar maple NRA).<br>Models were run with and without Time (i.e., sampling campaign) as fixed effect to detect temporal variability. Model structure took into account the unequally spaced repeated sampling in time (repeated measures; Time was specified as the number of months since the first campaign) by employing spatial power law covariance structure. In addition, the spatial relationship of plot pairs was included as random effect. NRA values (NRA+0.0001) required square-root transformation. |
| 2. Summer Herb-layer Foliage NRA | WS<br>M<br>WSxM | NRA values per species were averaged across all three sampling campaigns to minimize missing data points.<br>Data comprised nine herb-layer species; factor Species was included as random effect. Analyses were conducted with a) watershed pairs (requiring square-root transformation of (NRA+0.0001) and b) all four watersheds in same model for greater statistical power to detect a Maple effect (no transformation necessary).<br>In addition, NRA of each individual understory species was analyzed across all four watersheds.<br>All models used a factorial design, i.e., a) 2 WS × 2 M or b) 4 WS × 2 M, because herb-layer species were not always present under both the red and sugar maple of a plot pair, thus precluding the use of plot pair as random effect. |
| 3. Root NRA in fertilized WS3 and unfertilized WS7 | WS (i.e., fertilization)<br>Species<br>WS×Species | Dataset containing root NRA of plot-center red and sugar maple and four herb-layer species (no transformation necessary) was analyzed with a factorial ANOVA (2 WS × 6 Species). |
| 4. Root vs. Foliage NRA in fertilized WS3 and unfertilized WS7 | WS (i.e., fertilization)<br>Organ<br>WS×Organ | Root and foliage NRA was collected in midsummer 2019 (Table 1). A factorial ANOVA was conducted for each species separately. Factor Organ refers to foliage or root tissue. Square root (NRA + 0.0001) transformation was necessary for blackberry, ferns, and sugar maple, but not for common greenbrier, red maple, and violets. For foliage NRA, different fern species were combined into "ferns" to obtain a sufficient sample size for statistical analysis. |
| 5. Spring vs. Summer Foliage NRA of the | WS (i.e., fertilization) | Herb-layer spring foliar NRA was compared to the foliar NRA of all three summer campaigns in WS3 and WS7 for the following species: blackberry, common |

| | | |
|---|---|---|
| Herb-layer in fertilized WS3 and unfertilized WS7 | Time<br>WS×Time | greenbrier, star chickweed, and violets. A square-root transformation (NRA + 0.0001) was applied. In this repeated-measures (with unequal spacing) ANOVA, Time (identifying the sampling campaign) was specified as the number of months since the first foliage collection campaign in summer of 2018. Site was included as a random effect. |
| 6. NRAA | | The same model design structure was used as for the NRA analyses. |

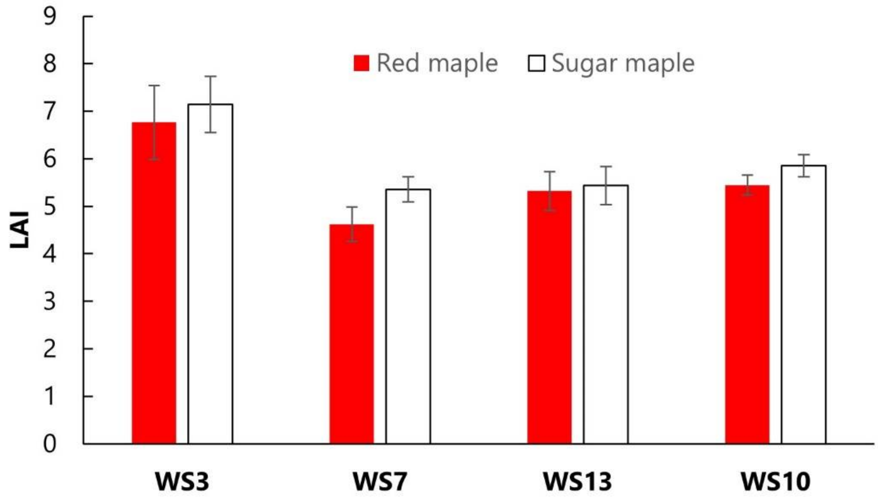


Figure S1. LAI under plot-center red maple and sugar maples in each watershed at the Fernow Experimental Forest, West Virginia, USA (*n* = 9 per watershed and maple species; the value per plot-center tree was derived from the mean of four measurements taken at the cardinal directions facing the tree at a distance of 1.75 m from the stem).

Supplementary Methods – NRAA of herb-layer shoots

*A. Summer*

Understory above-ground NRAA was calculated for each species recorded in each plot (Equation S1) and then summed per plot:

$$Understory\ NRAA\ (\mu mol\ m^{-2}\ hr^{-1}) = biomass\ (g\ m^{-2})\ \times\ NRA\ (\mu mol\ g^{-1}\ h^{-1}) \qquad \text{(Equ. S1)}$$

Understory biomass was derived from cover-biomass relationships developed by Smith [43]. Equations for biomass were either 1) species-specific for any species that had $n > 10$ data points for creating the cover-biomass relationship or 2) based on functional groups (woody seedlings, herb, fern, shrub/vine) for all other species (Table S3). Biomass was calculated for each species in each plot. NRA values for species found in the plots were obtained by different methods depending on the NRA sample size for each species. A) For each of the nine most common herb-layer species (Table S3: NRA value method "A"), NRA values were averaged across plots for each watershed, overstory species, and sampling campaign (prior to averaging, NRA values had been ln-transformed for normality; the average was then back-transformed). For these nine species, the watershed-, overstory-, and campaign-specific values were input into Equation S1. If NRA values were not available for all sampling campaigns, data gaps were filled by using NRA values collected for this species at a different campaign but in the same watershed under the same overstory species. B) For infrequent species, for which $n > 10$ data points per species were available across all sampling campaigns, watersheds, and overstory species (Table S3: NRA value method "B"), the available (and ln-transformed) NRA values were averaged for each species, and the average was back-transformed. Then, this one, species-specific average NRA per species was applied to all sampling campaigns, watersheds, and overstory species. C) For the most infrequent of species for which either only a few ($n \leq 10$) or no direct NRA measurement were available, NRA values were assigned based on functional group. For example, there were 29 herb species, but a species-specific NRA was available only for 13 of them. To calculate "herb" functional group NRA, the average NRA values for each of the 13 herb species were first ln-transformed. The resulting 13 values were then averaged, the one average back-transformed, and applied to the 16 species for all sampling campaigns, watersheds, and overstory species (Table S3: NRA value method "C").

Table S4: Biomass equation type (1 – species-specific; 2 – based on functional group) and NRA value type (A – species-specific per watershed, overstory maple, and summer campaign; B – single, species-specific value applied to all watersheds, both overstory maples, and all summer campaigns; C – based on functional group) used for calculating herb-layer foliar NRA per area (NRAA) for each of the three summer campaigns. Further explanations of the biomass equation type and the methods for obtaining NRA values are given in the text above.

| Functional type | Species | Biomass equation type* | NRA value type** |
|---|---|---|---|
| Woody seedling | *Acer pensylvanicum* | 1 | A |
| Woody seedling | *Acer rubrum* | 1 | B |
| Woody seedling | *Acer saccharum* | 1 | B |
| Woody seedling | *Fraxinus americana* | 1 | B |

| | | | |
|---|---|---|---|
| Woody seedling | *Magnolia acuminata* | 2 | B |
| Woody seedling | *Quercus rubra* | 1 | B |
| Woody seedling | Other | 2 | C |
| Herbs | *Disporum lanuginosum* | 1 | C |
| Herbs | *Medeola virginiana* | 2 | B |
| Herbs | *Stellaria pubera* | 1 | A |
| Herbs | *Viola* spp. | 1 | A |
| Herbs | Other | 2 | C |
| Ferns | *Dennstaedtia punctilobula* | 1 | A |
| Ferns | *Dryopteris intermedia* | 1 | A |
| Ferns | *Polystichum acrostichoides* | 1 | A |
| Ferns | *Thelypteris noveboracensis* | 1 | A |
| Ferns | Other | 2 | C |
| Shrub/vine | *Rubus* spp. | 1 | A |
| Shrub/vine | *Smilax rotundifolia* | 1 | A |
| Shrub/vine | Other | 2 | C |

*Cover – as the basis for biomass estimation – was determined in 2018 and once in 2019
** NRA was measured once in 2018 and twice in 2019

### *B. Spring NRAA in fertilized WS3 and unfertilized WS7*

Calculation of spring above-ground herb NRAA also used Equation S1. Spring herb-layer biomass estimation was identical to the method used for the summer campaigns (described above). NRA values for each species found in the plots in spring were determined by methods that differed from summer; this was due to a smaller subset of species being collected in only two watersheds (WS3, WS7) and without association to an overstory maple species. For each of the four most common herb-layer species, species-specific NRA values were averaged across plots for each watershed (prior to averaging, NRA values were transformed as above for summer herb-layer NRAA) (Table S4, NRA value method X). For each of these four species, the watershed-specific values were input into Equation S1. For most, infrequent species for which either only a few ($n \leq 10$) or no direct NRA measurement were available in spring, NRA values were assigned based on functional group (herb or shrub/vine). Herb functional group NRA was based mostly on the species from WS7, because in WS3 the herb trillium ($n = 1$) was the only one encountered in addition to star chickweed and violets (Figure S2). Since these two species reacted strongly to fertilization, the more conservative herb functional group value from WS7 was applied to WS3 which may have resulted in underestimating WS3 herb NRAA. To calculate WS7-herb functional group NRA, the average NRA values for each of the eight herb species measured (except fairy bells with $n = 1$; Figure S2) were first ln-transformed and then averaged to avoid bias towards more frequent species. The resulting average was back-transformed and applied to herb species (Table S4, NRA value method Y*) in both watersheds. To calculate the "shrub/vine" functional group NRA, the average NRA values for the two shrub/vine species measured (blackberry, common greenbrier) were first ln-transformed then averaged. The average was back-transformed and applied to the shrub/vine species for each watershed (Table S4, NRA value method Y). For species in the fern and woody seedling functional groups, functional group NRA values from the summer campaigns were used as no spring NRA was collected (Figure S2).

Table S5. Biomass equation type ((1 – species-specific; 2 – based on functional group) and NRA value type (X – species-specific and specific to watershed (WS) 3 or WS7; Y – based on functional group and specific to WS3 or WS7) used for calculating herb-layer foliar NRA per area (NRAA) for the spring campaign (May 2019) in fertilized WS3 and unfertilized WS7. Further explanations of the biomass equation type and the methods for obtaining NRA values are given in the text above.

| Functional type | Species | Biomass equation type | NRA value |
|---|---|---|---|
| Woody seedling | | 1 or 2 | From summer campaigns |
| Ferns | | 1 or 2 | From summer campaigns |
| Herbs | *Stellaria pubera* | 1 | X |
| Herbs | *Viola* spp. | 1 | X |
| Herbs | Other | 1 or 2 | Y* |
| Shrub/vine | *Rubus* spp. | 1 | X |
| Shrub/vine | *Smilax rotundifolia* | 1 | X |
| Shrub/vine | Other | 2 | Y |

*"Herb" functional group NRA values are mostly from WS7 only, since species common to WS7 were not encountered in WS3 (except for trillium). This may underestimate herb functional group NRAA of WS3.

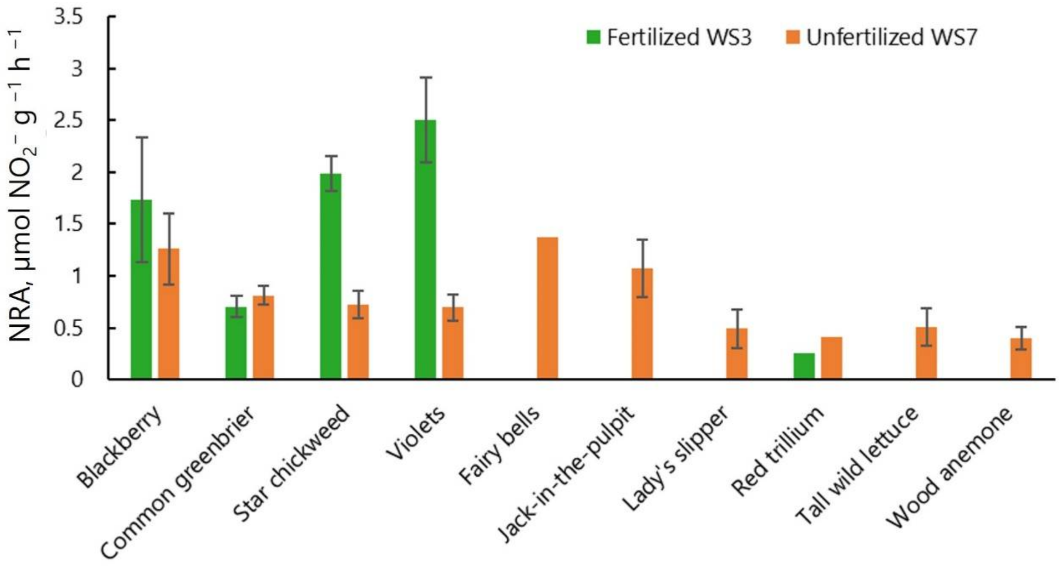


Figure S2. Foliar NRA of spring herb species in fertilized watershed (WS) 3 and unfertilized WS7 in the 2019 spring campaign. Error bars represent 1 SE, based on $n$ = 2–9 (maximum $n$ = 9 sites; sampling locations were not associated with particular overstory maple due to sparse vegetative cover at the time of sampling). Scientific names of species are given in Table S1.

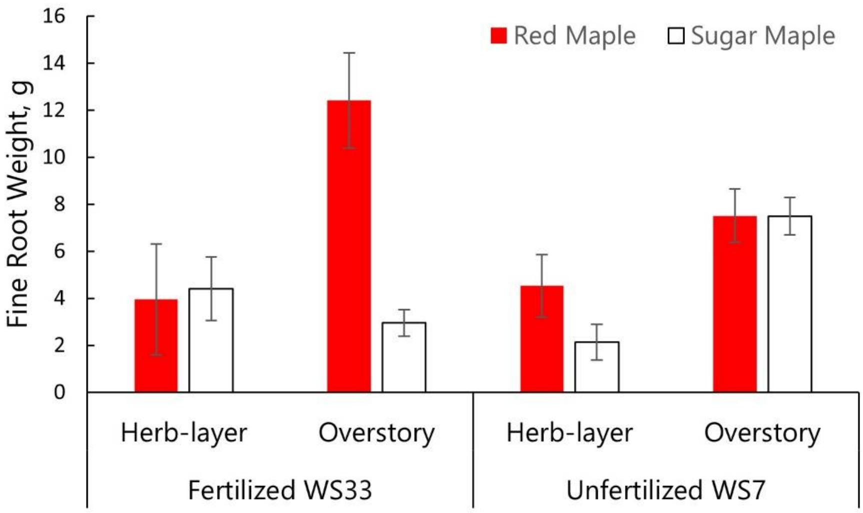


Figure S3. Biomass of herb-layer fine roots under red maple and under sugar maple trees, and fine root biomass of the overstory maples themselves. Root weight represents the fine roots collected from the top 10 cm in an area of 0.14 $m^2$ from sites in fertilized watershed (WS) 3 and unfertilized WS7 at the Fernow Experimental Forest, USA, during the midsummer 2019 campaign. Error bars represent 1 SE, based on $n$ = 2–9 (maximum $n$ = 9 sites).