Supplementary Material

Drought Deteriorates the N Stoichiometry of Biomass Production in European Beech Saplings Under Global Change

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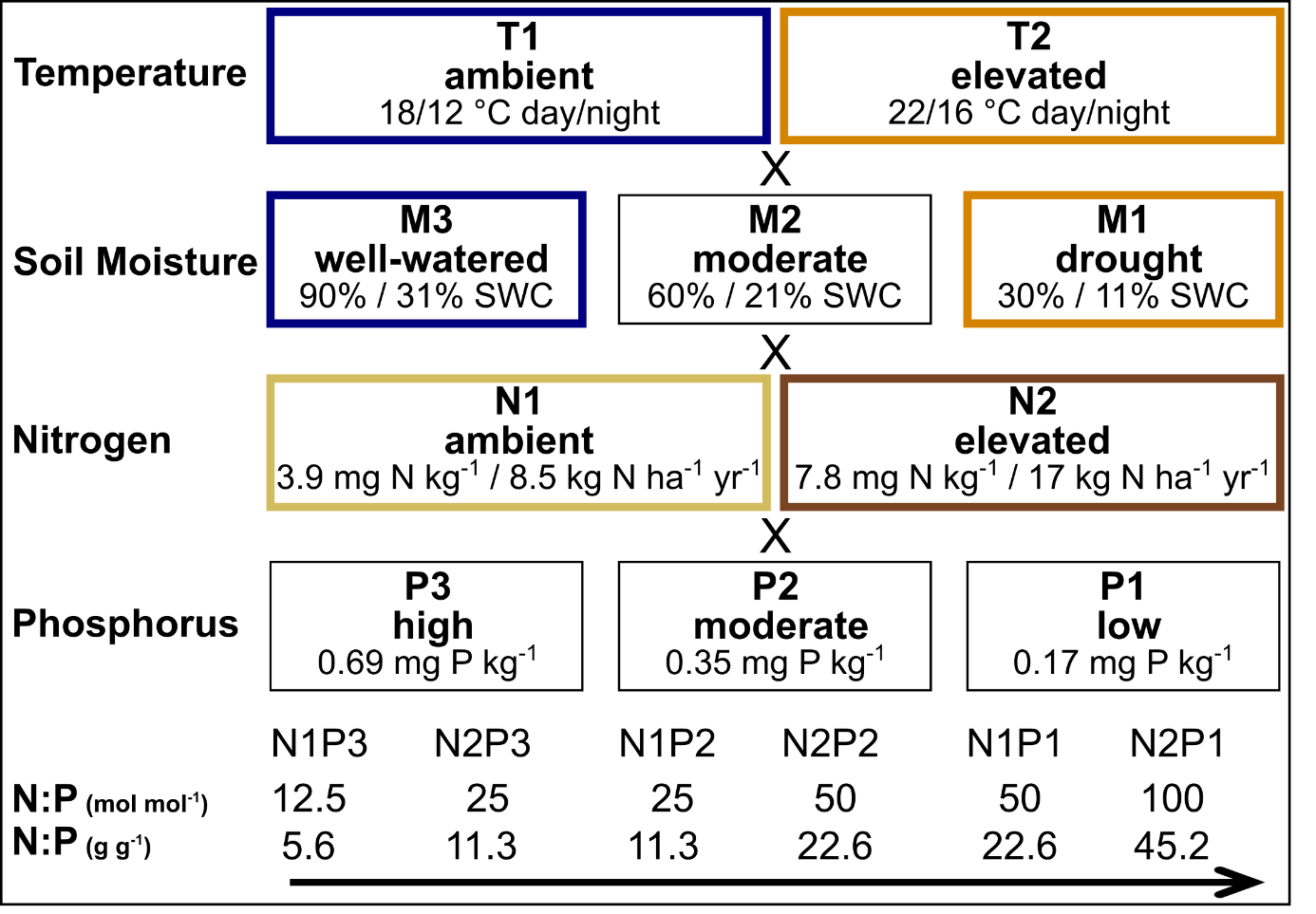
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# Supplementary Tables

**Supplementary Table S1.** Study design of the full-factorial experiment with two temperature, three soil moisture, two N supply and three P supply levels (*n* = 36 treatments in total; after Köhler et al., 2018). A gradient from current environmental conditions to the expected future global change conditions (elevated temperature and N deposition and reduced soil moisture and P availability) was simulated. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered. N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high.



**Supplementary Table S2.** Means and SE of photosynthetic capacity (Amax), total C assimilation, and ectomycorrhizal fungal (ECMF) colonization rate of European beech saplings grown at increasing soil N:P ratios and ambient (ambient temperature and soil moisture, AmbT+AmbM; treatment T1M3) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM; treatment T2M1) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (*n* = eight replicates per treatment for ECMF colonization; *n* = five replicates per treatment for Amax and total C assimilation). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. n/d = no data.

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| **Treatment** | |  | **Amax (µmol CO2 m-2 s-1)** |  | **Total C assimilation (µmol C h-1)** |  | **ECMF colonization rate (%)** |
| AmbT+AmbM | N1P3 |  | 3.2 (0.3) ab |  | 60.4 (8.9) bc |  | 80.6 (10.7) ab |
|  | N2P3 |  | 4.3 (0.4) b |  | 100.5 (9.4) c |  | 69.1 (8.1) ab |
|  | N1P2 |  | n/d |  | n/d |  | 72.6 (14.0) ab |
|  | N2P2 |  | n/d |  | n/d |  | 83.6 (7.5) b |
|  | N1P1 |  | 1.8 (0.3) a |  | 54.9 (11.4) bc |  | 71.3 (4.2) ab |
|  | N2P1 |  | 2.2 (0.2) a |  | 53.3 (9.2) bc |  | 80.4 (7.3) ab |
|  |  |  |  |  |  |  |  |
| ElevT+RedM | N1P3 |  | 2.1 (0.3) a |  | 13.2 (6.5) ab |  | 30.5 (9.2) a |
|  | N2P3 |  | 2.0 (0.3) a |  | 5.7 (0.2) a |  | 40.9 (20.2) ab |
|  | N1P2 |  | n/d |  | n/d |  | 57.7 (10.7) ab |
|  | N2P2 |  | n/d |  | n/d |  | 47.1 (11.0) ab |
|  | N1P1 |  | 2.5 (0.5) ab |  | 28.2 (10.4) ab |  | 47.4 (24.1) ab |
|  | N2P1 |  | 1.9 (0.6) a |  | 1.6 (0.1) a |  | 22.5 (16.5) ab |

**Supplementary Table S3.** Means and SE of total, leaf, coarse root, and fine root biomass of European beech saplings grown at increasing soil N:P ratios and ambient (ambient temperature and soil moisture, AmbT+AmbM; treatment T1M3) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM; treatment T2M1) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (*n* = eight replicates per treatment). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Biomass (g)** | | | | | | |
| **Treatment** | |  | **Total** |  | **Leaves** |  | **Coarse roots** |  | **Fine roots** |
| AmbT+AmbM | N1P3 |  | 7.6 (1.1) cd |  | 0.9 (0.2) bcd |  | 2.7 (0.4) b |  | 2.3 (0.4) ab |
|  | N2P3 |  | 8.7 (0.7) d |  | 1.0 (0.1) cd |  | 2.8 (0.3) b |  | 2.6 (0.3) b |
|  | N1P2 |  | 7.8 (1.1) cd |  | 1.0 (0.2) cd |  | 2.7 (0.4) b |  | 2.2 (0.3) ab |
|  | N2P2 |  | 8.3 (0.8) d |  | 1.0 (0.1) cd |  | 2.8 (0.2) b |  | 2.5 (0.4) ab |
|  | N1P1 |  | 8.5 (0.7) d |  | 1.2 (0.1) d |  | 2.9 (0.2) b |  | 2.2 (0.3) ab |
|  | N2P1 |  | 6.5 (0.5) bcd |  | 0.9 (0.1) bcd |  | 2.2 (0.3) b |  | 1.8 (0.2) ab |
|  |  |  |  |  |  |  |  |  |  |
| ElevT+RedM | N1P3 |  | 3.4 (0.3) a |  | 0.4 (0.1) ab |  | 0.8 (0.1) a |  | 1.3 (0.1) a |
|  | N2P3 |  | 3.9 (0.3) ab |  | 0.5 (0.1) abc |  | 0.8 (0.1) a |  | 1.4 (0.2) ab |
|  | N1P2 |  | 3.8 (0.5) ab |  | 0.5 (0.1) abc |  | 1.1 (0.2) a |  | 1.4 (0.2) ab |
|  | N2P2 |  | 3.6 (0.3) a |  | 0.3 (0.1) a |  | 1.1 (0.1) a |  | 1.3 (0.1) a |
|  | N1P1 |  | 3.9 (0.4) ab |  | 0.5 (0.1) abc |  | 1.1 (0.1) a |  | 1.3 (0.2) a |
|  | N2P1 |  | 4.4 (0.6) abc |  | 0.4 (0.2) ab |  | 1.1 (0.1) a |  | 1.5 (0.3) ab |

**Supplementary Table S4.** Means and SE of N uptake efficiency (NUptakeE), photosynthetic N use efficiency (PNUE), and N use efficiency (NUE) of European beech saplings grown at increasing soil N:P ratios and ambient temperature and reduced soil moisture (AmbT+RedM; treatment T1M1) or elevated temperature and ambient soil moisture (ElevT+AmbM; treatment T2M3) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (*n* = eight replicates per treatment). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. n/d = no data.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | |  | **NUptakeE(g NDW g-1 Nadded)** |  | **PNUE (µmol CO2 g-1 Ns-1)** |  | **NUE (g DW g-1 NDW)** |
| AmbT+RedM | N1P3 |  | 8.2 (0.7) cde |  | 4.4 (1.0) a |  | 57.8 (4.0) a |
|  | N2P3 |  | 4.2 (0.2) ab |  | 3.1 (0.4) a |  | 47.2 (2.0) a |
|  | N1P2 |  | 7.7 (1.0) bcd |  | n/d |  | 56.8 (8.8) a |
|  | N2P2 |  | 4.0 (0.3) ab |  | n/d |  | 66.3 (3.6) abc |
|  | N1P1 |  | 9.1 (0.6) cde |  | 3.5 (0.2) a |  | 62.0 (4.8) ab |
|  | N2P1 |  | 4.1 (0.3) a |  | 3.8 (0.7) a |  | 69.1 (5.1) abc |
|  |  |  |  |  |  |  |  |
| ElevT+AmbM | N1P3 |  | 10.3 (1.1) de |  | 8.4 (1.1) a |  | 100.9 (8.9) cd |
|  | N2P3 |  | 5.7 (0.6) abc |  | 7.5 (0.5) a |  | 94.7 (10.6) bcd |
|  | N1P2 |  | 11.8 (1.2) de |  | n/d |  | 119.1 (9.3) d |
|  | N2P2 |  | 5.9 (0.4) abc |  | n/d |  | 106.3 (8.4) cd |
|  | N1P1 |  | 12.5 (1.1) e |  | 5.9 (1.7) a |  | 104.2 (11.5) cd |
|  | N2P1 |  | 4.5 (0.8) ab |  | 5.1 (1.0) a |  | 81.8 (6.8) abcd |

**Supplementary Table S5.** Means and SE of N uptake efficiency (NUptakeE), photosynthetic N use efficiency (PNUE), and N use efficiency (NUE) of European beech saplings with increasing temperature, soil moisture, P availability, and N availability in climate chambers. n/d = no data.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Effect** | **Levels** | **NUptakeE(g NDW g-1 Nadded)** | **PNUE (µmol CO2 g-1 Ns-1)** | **NUE (g DW g-1 NDW)** |
| Temperature | T1 | 6.8 (0.3) | 4.3 (0.3) | 87.7 (2.5) |
|  | T2 | 7.6 (0.3) | 5.2 (0.6) | 79.2 (2.8) |
| Moisture | M1 | 6.2 (0.3) | 3.4 (0.3) | 53.0 (1.8) |
|  | M2 | 7.7 (0.4) | n/d | 93.5 (2.7) |
|  | M3 | 7.7 (0.4) | 5.4 (0.4) | 101.9 (2.6) |
| P availability | P1 | 7.1 (0.4) | 3.8 (0.3) | 77.5 (2.9) |
|  | P2 | 7.1 (0.3) | n/d | 90.9 (3.4) |
|  | P3 | 7.4 (0.3) | 5.5 (0.4) | 82.0 (3.3) |
| N availability | N1 | 9.5 (0.3) | 4.5 (0.4) | 85.1 (2.9) |
|  | N2 | 4.9 (0.1) | 4.6 (0.4) | 81.9 (2.4) |

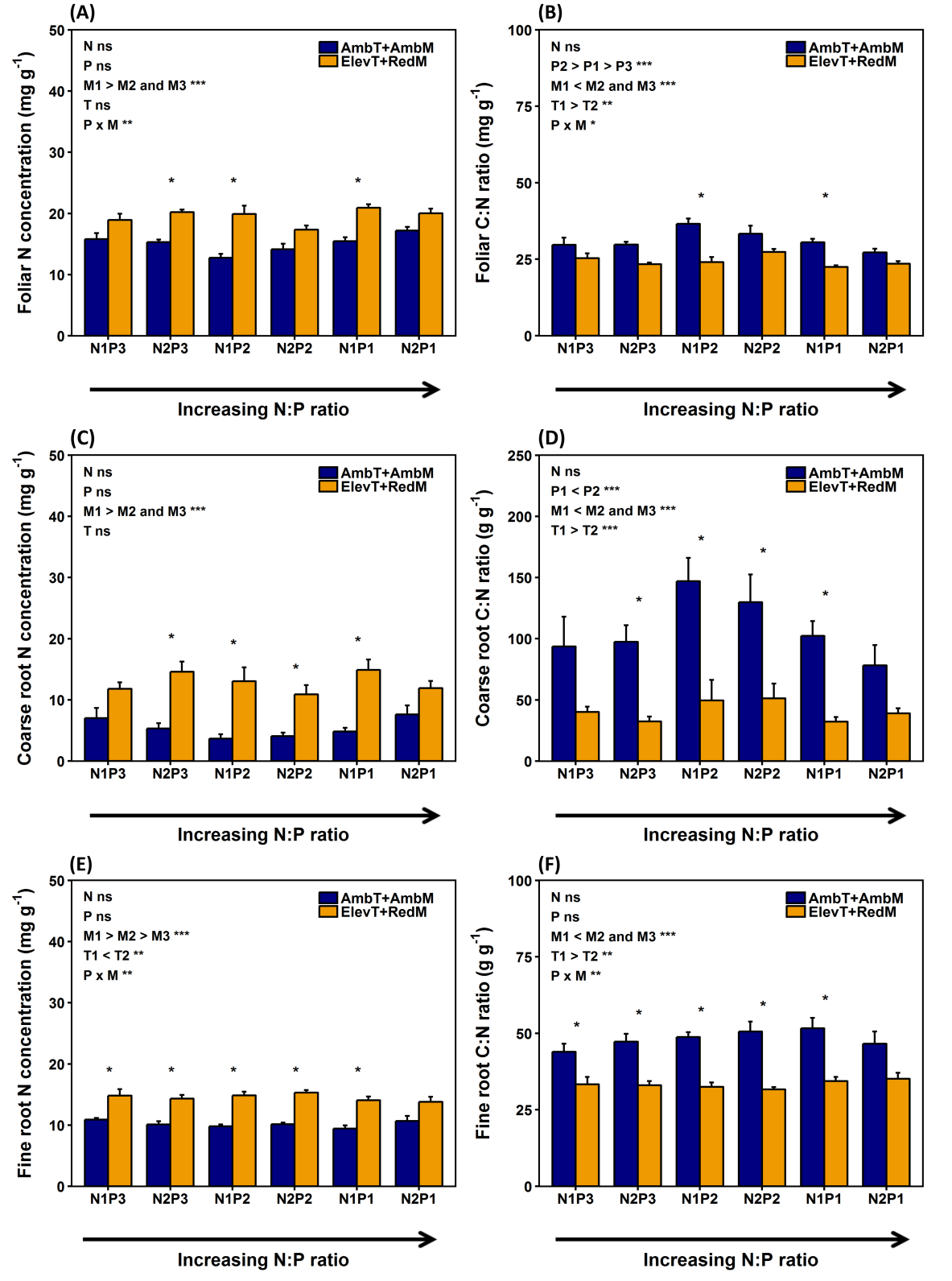
**Supplementary Table S6.** Means and SE of foliar, coarse root, and fine root N:P ratios of European beech saplings grown at increasing soil N:P ratios and ambient (ambient temperature and soil moisture, AmbT+AmbM; treatment T1M3) or climate change conditions (elevated temperature and reduced soil moisture, ElevT+RedM; treatment T2M1) in climate chambers. Four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey were calculated using a dataset limited to temperature and soil moisture treatments only. Significant differences between soil N:P treatments are indicated by different lower-case letters (*n* = eight replicates per treatment). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **N:P ratio (g g-1)** | | | | |
| **Treatment** | |  | **Leaves** |  | **Coarse roots** |  | **Fine roots** |
| AmbT+AmbM | N1P3 |  | 8.2 (1.3) ab |  | 4.8 (0.6) ab |  | 6.3 (0.2) a |
|  | N2P3 |  | 6.0 (0.5) a |  | 3.9 (0.5) a |  | 6.1 (0.3) a |
|  | N1P2 |  | 11.6 (0.7) bcd |  | 4.9 (0.6) ab |  | 8.1 (0.3) ab |
|  | N2P2 |  | 11.0 (1.2) bc |  | 6.1 (1.2) ab |  | 8.5 (0.7) ab |
|  | N1P1 |  | 30.7 (1.4) f |  | 70.0 (40.4) def |  | 18.1 (3.6) cd |
|  | N2P1 |  | 29.5 (2.6) ef |  | 29.2 (6.9) def |  | 19.9 (2.7) d |
|  |  |  |  |  |  |  |  |
| ElevT+RedM | N1P3 |  | 20.8 (2.9) ef |  | 10.0 (2.3) abc |  | 10.3 (1.1) b |
|  | N2P3 |  | 19.1 (1.1) def |  | 11.0 (2.1) bcd |  | 9.6 (1.1) ab |
|  | N1P2 |  | 17.4 (1.2) cde |  | 9.6 (1.5) abc |  | 10.4 (0.5) b |
|  | N2P2 |  | 18.9 (0.8) def |  | 18.0 (4.5) cde |  | 11.8 (0.9) bc |
|  | N1P1 |  | 32.1 (5.2) ef |  | 41.8 (6.7) f |  | 20.4 (1.8) d |
|  | N2P1 |  | 31.5 (2.0) f |  | 36.8 (4.6) ef |  | 21.3 (0.9) d |

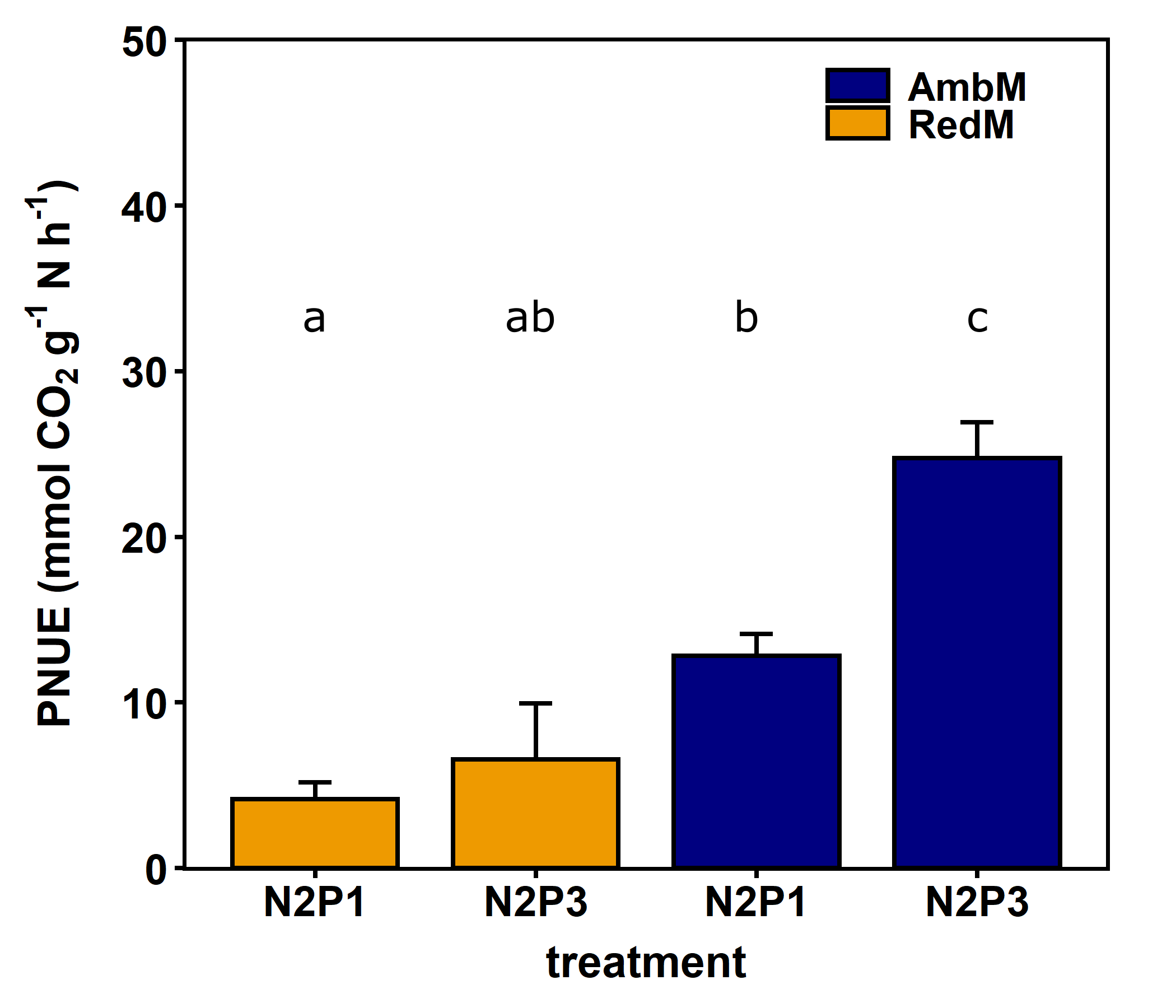
**Supplementary Table S7.** Pearson’s correlations between the ectomycorrhizal fungal (ECMF) community and plant biomass, assimilation, nutrient concentrations, and nutrient ratios of European beech saplings grown at increasing soil N:P ratios and climate change. Given are the correlation coefficients *R* and the probabilities of error *P* (\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001; *n* = 36 treatments). Significant correlations are indicated by bold letters.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **ECMF**  **colonization rate** | **ECMF**  **species richness** | **ECMF**  **α diversity** |
| Leaf biomass | **0.39\*** | 0.27 | **0.36\*** |
| Total C assimilation | 0.23 | 0.22 | 0.31 |
|  |  |  |  |
| C leaves | -0.30 | **-0.52\*\*** | **-0.44\*\*** |
| N leaves | **-0.42\*** | **-0.39\*** | **-0.37\*** |
| P leaves | 0.01 | 0.16 | 0.06 |
| C:N leaves | **0.40\*** | **0.36\*** | **0.36\*** |
| N:P leaves | -0.12 | -0.20 | -0.15 |
|  |  |  |  |
| Coarse root biomass | **0.42\*** | **0.37\*** | **0.44\*\*** |
| C coarse roots | **0.51\*\*** | **0.34\*** | 0.07 |
| N coarse roots | **-0.51\*\*** | **-0.43\*\*** | **-0.37\*** |
| P coarse roots | -0.17 | -0.13 | -0.17 |
| C:N coarse roots | **0.49\*\*** | **0.38\*** | 0.31 |
| N:P coarse roots | -0.05 | -0.04 | 0.00 |
|  |  |  |  |
| Fine root biomass | **0.40\*** | **0.44\*\*** | **0.49\*\*** |
| C fine roots | 0.26 | **0.37\*** | 0.28 |
| N fine roots | **-0.44\*\*** | **-0.34\*** | **-0.35\*** |
| P fine roots | -0.12 | -0.03 | -0.04 |
| C:N fine roots | **0.48\*\*** | 0.32 | 0.30 |
| N:P fine roots | -0.08 | -0.11 | -0.09 |

# Supplementary Figures



**Supplementary Figure S1.** Means and SE of foliar, coarse root, and fine root **(A, C, E)** N concentration and **(B, D, F)** C:N ratios of European beech saplings grown at increasing soil N:P ratios and ambient (blue bars; ambient temperature and soil moisture, AmbT+AmbM) or climate change conditions (orange bars; elevated temperature and reduced soil moisture, ElevT+RedM) in climate chambers. The associated tables show the results of four-factorial ANOVAs and post-hoc multiple comparisons according to Tukey across all experimental treatments (\**P* < 0.05, \*\**P* < 0.01, \*\*\**P*< 0.001; ns, not significant; *n* = eight replicates per treatment). Asterisks show significant differences between ambient and climate change conditions (\**P* < 0.05). N supply: N1, ambient; N2, elevated. P supply: P1, low; P2, moderate; P3, high. Temperature: T1, ambient; T2, elevated. Soil moisture: M1, drought; M2, moderately moist; M3, well-watered.



**Supplementary Figure S2.** Response of photosynthetic N use efficiency (PNUE; means and SE) of European beech saplings to factorial addition of phosphorus and soil moisture (*n* = 16 replicates per treatment). The response represents super-additive independent co-limitation (as defined by Harpole et al. 2011), with a dominating effect exerted by soil drought and a subordinate effect by P deficiency. Significant differences between soil N:P treatments are indicated by different lower-case letters. N supply: N1, ambient; N2, elevated. P supply: P1, low; P3, high. Soil moisture: RedM, drought (orange bars; treatment M1); AmbM, ambient, well-watered (blue bars; treatment M3).