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Global variability of leaf functional trait under nitrogen and phosphorus co-limitation

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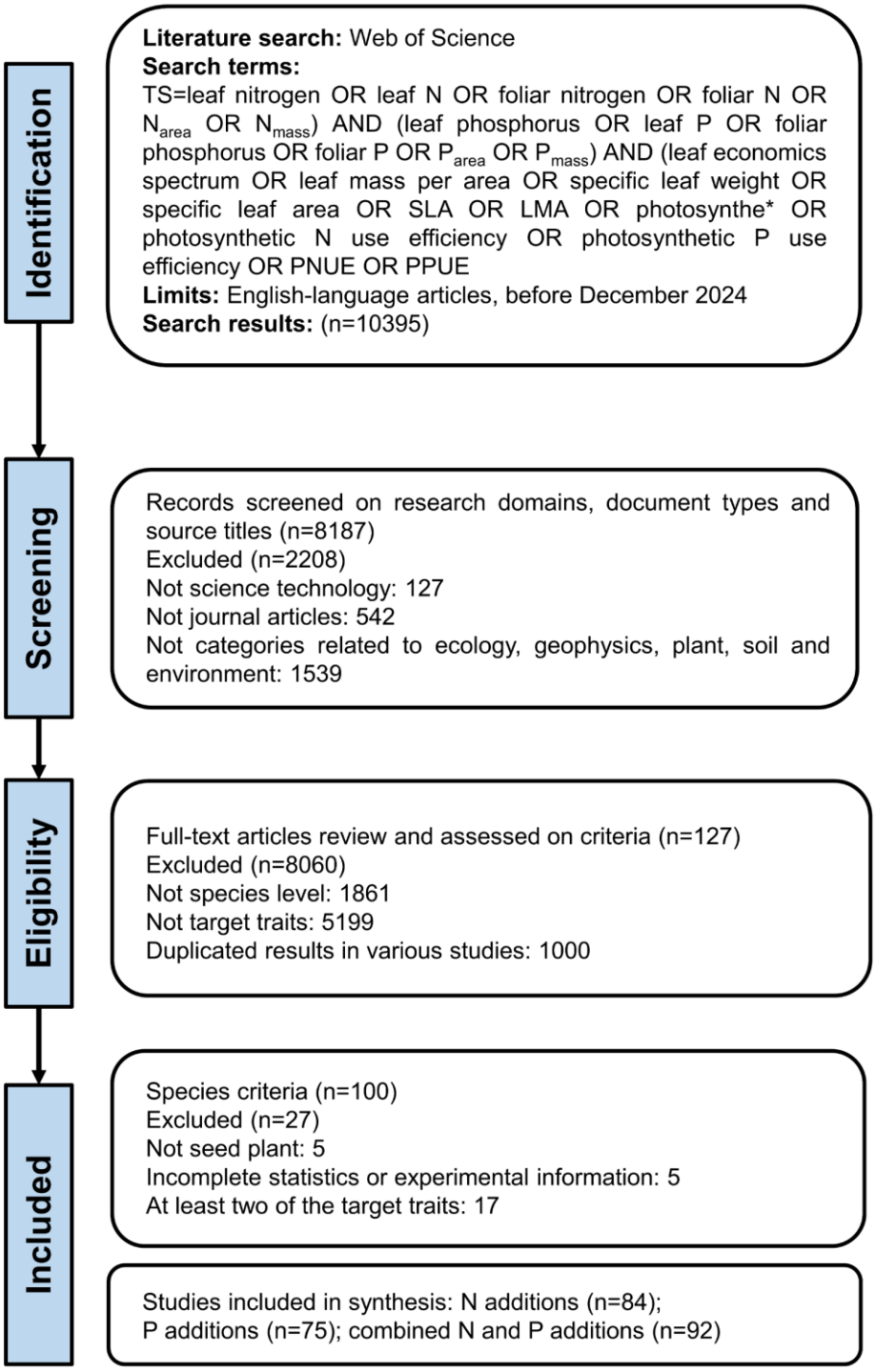
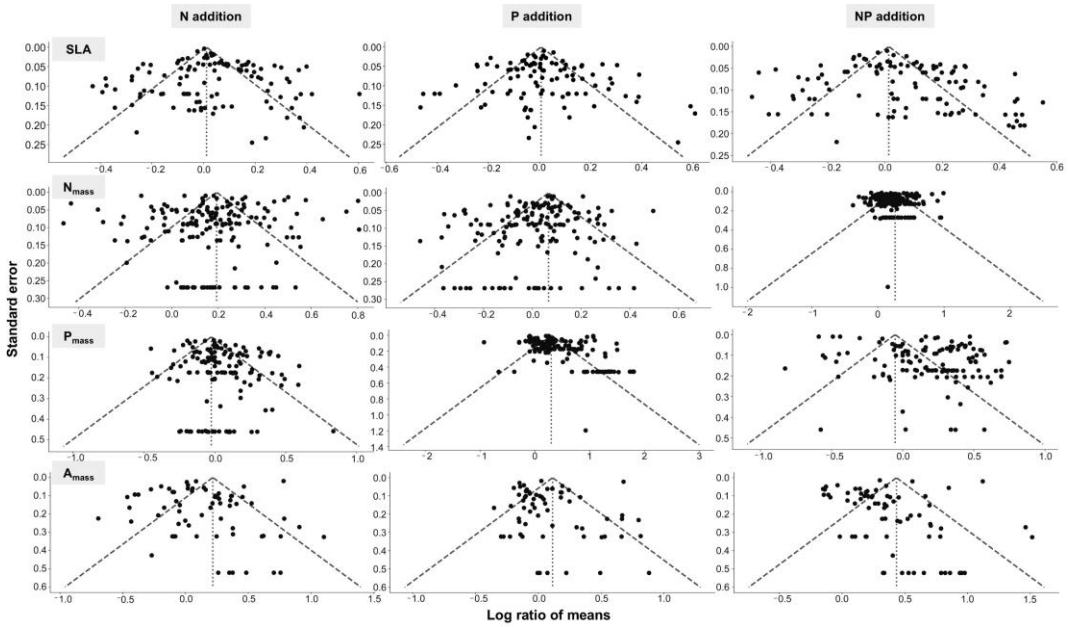
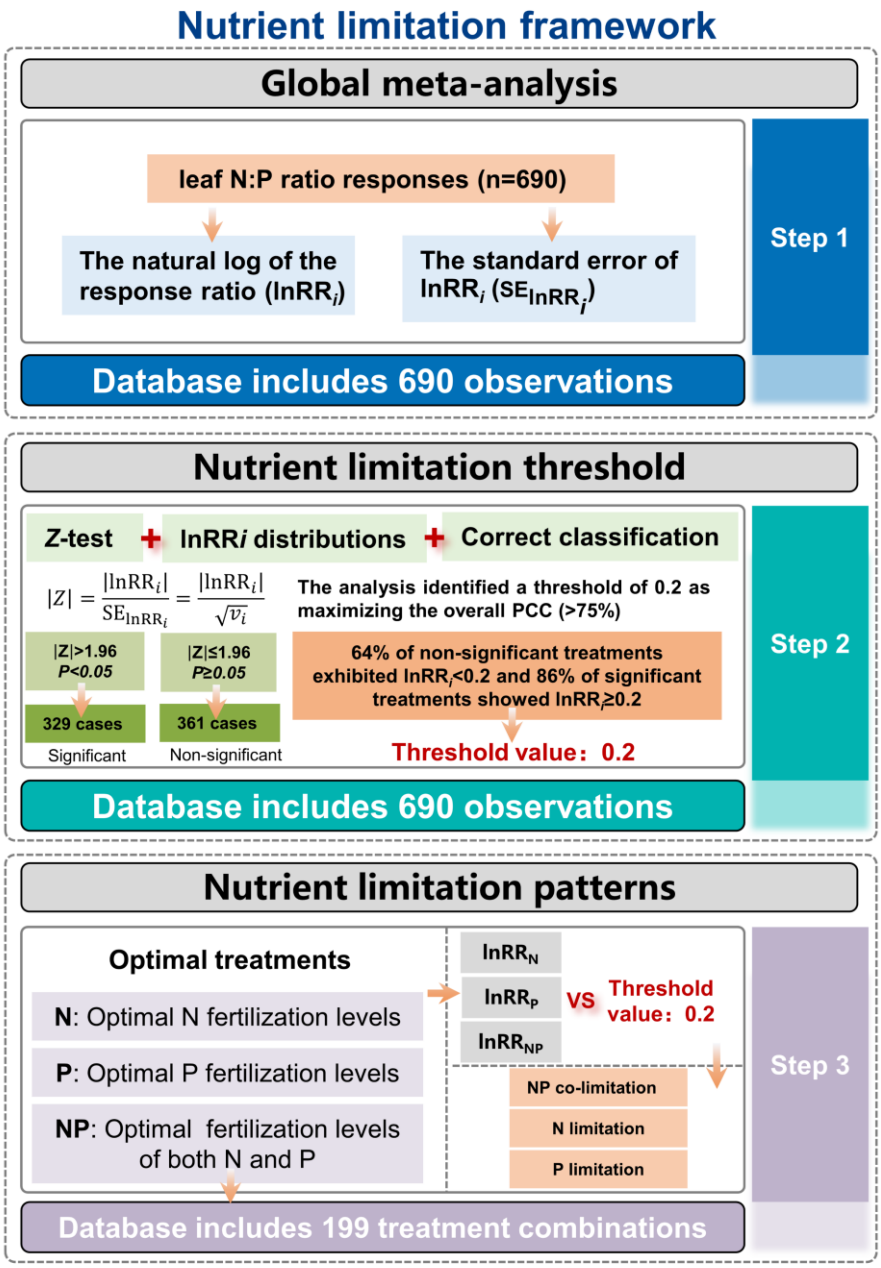


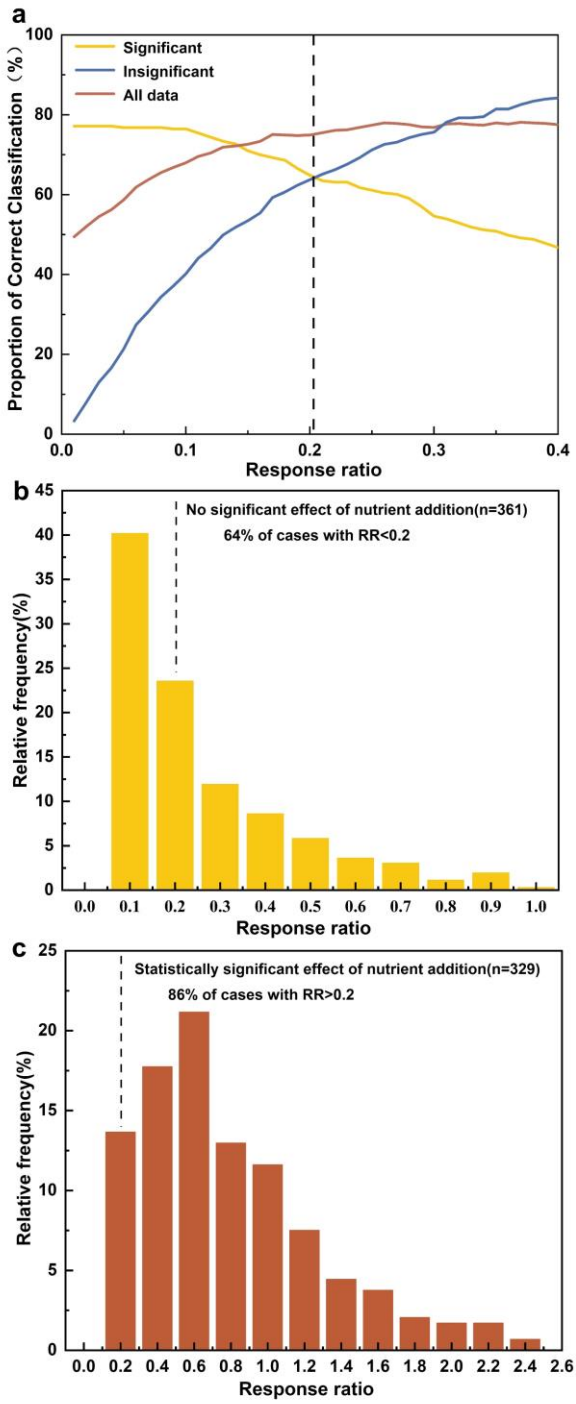
Supplementary Fig. 1 The PRISMA flow diagram for the selection of published papers.



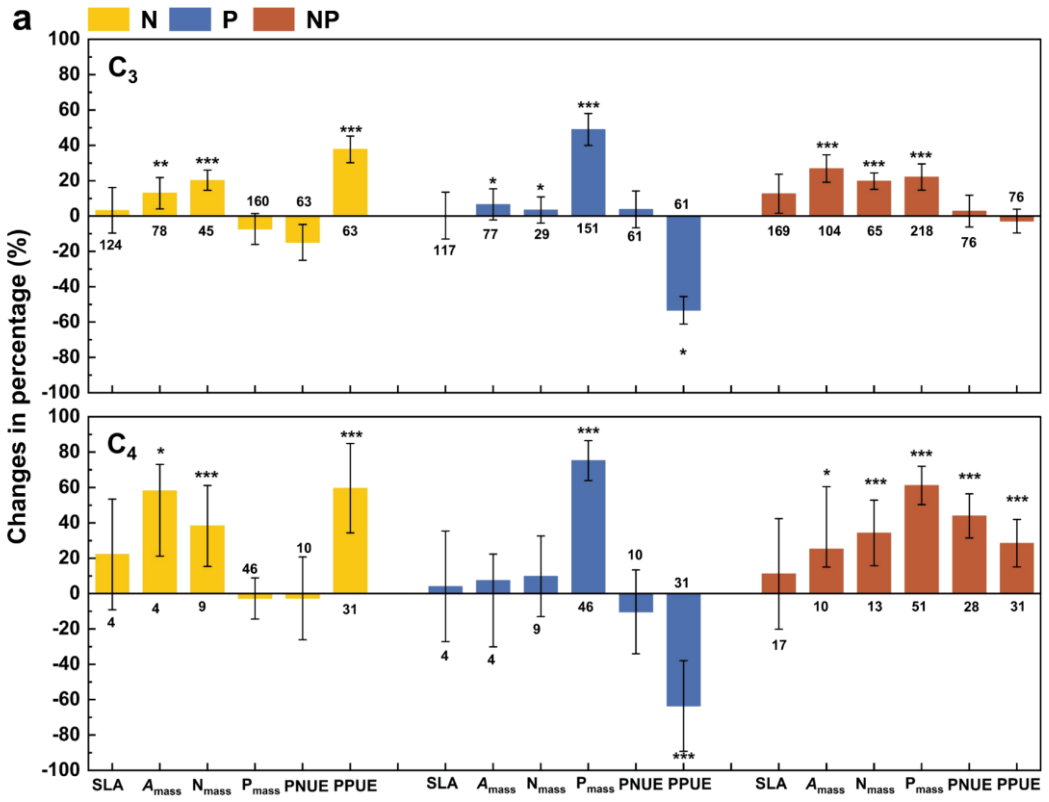
Supplementary Fig. 2 Funnel plots showing the effects of nitrogen (N), phosphorus (P), and NP addition on plant leaf functional traits. The *x*-axis represents the log response ratio, where positive values indicate higher trait values in the treatment relative to the control, and negative values indicate lower trait values. The *y*-axis represents the standard error (SE) of the effect size, with smaller SE (upper part of the plot) indicating more precise estimates. The vertical dotted lines denote the mean effect size, and the diagonal dashed lines represent the 95% confidence interval.

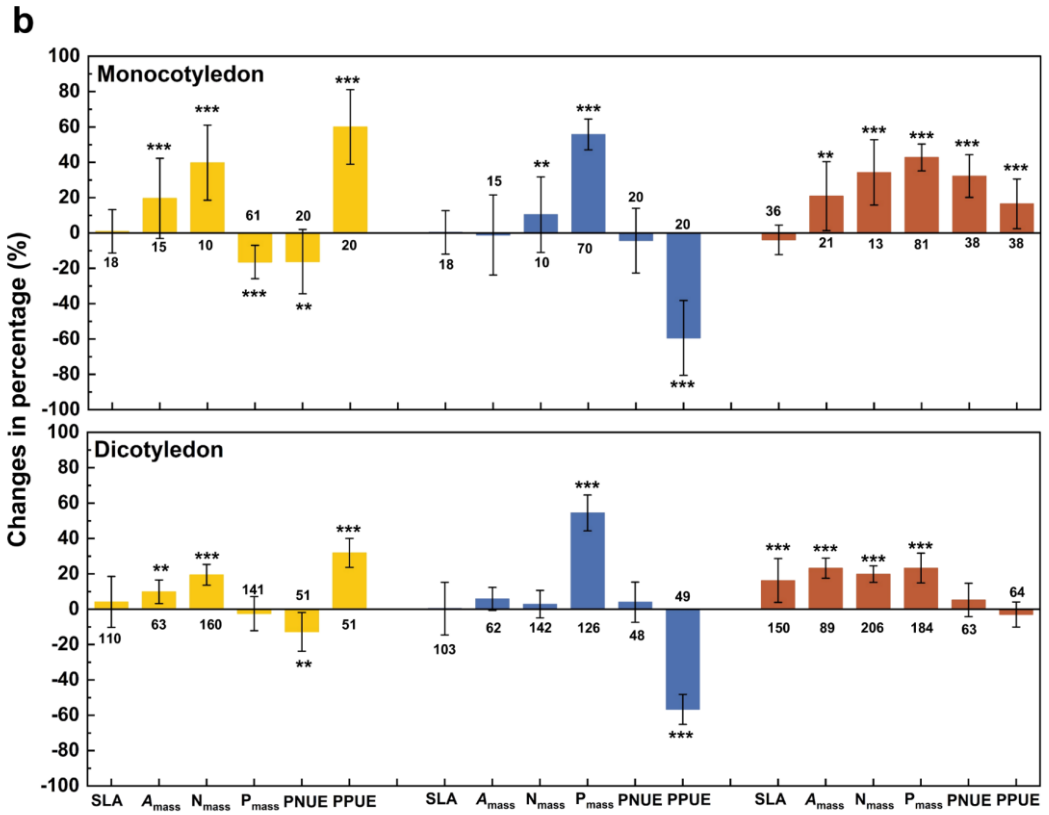


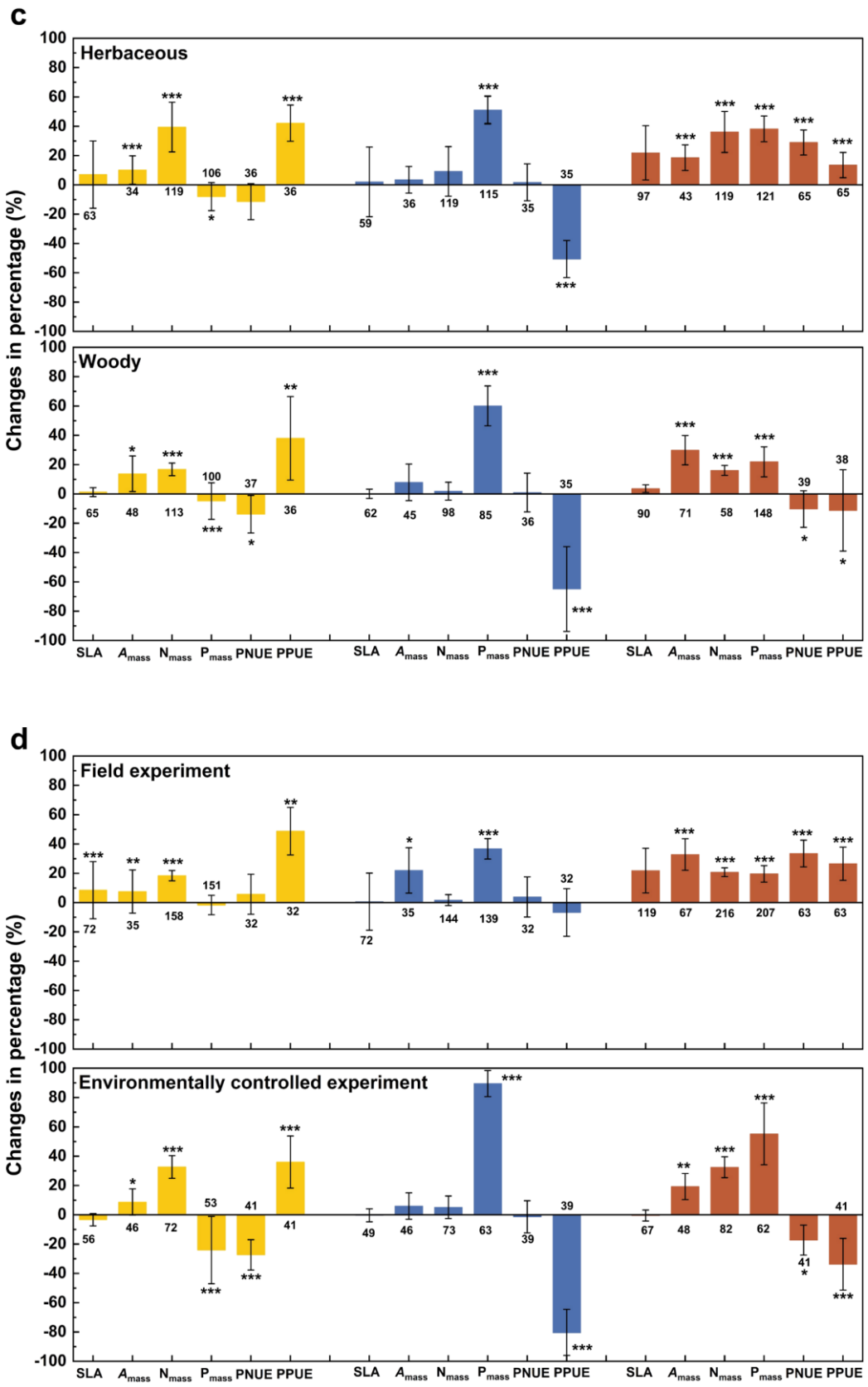
Supplementary Fig. 3 The flow diagram of nutrient limitation framework.



Supplementary Fig. 4 Distribution of values of relative response ratio of nitrogen (N): phosphorus (P). a, The proportion of correct classification for the threshold values dividing N and P limitations. **b**, The results of the treatments which were reported in publications to be non-significantly higher than the control treatment. **c**, The results of the treatments which were reported in publications to be significantly higher than the control treatment.

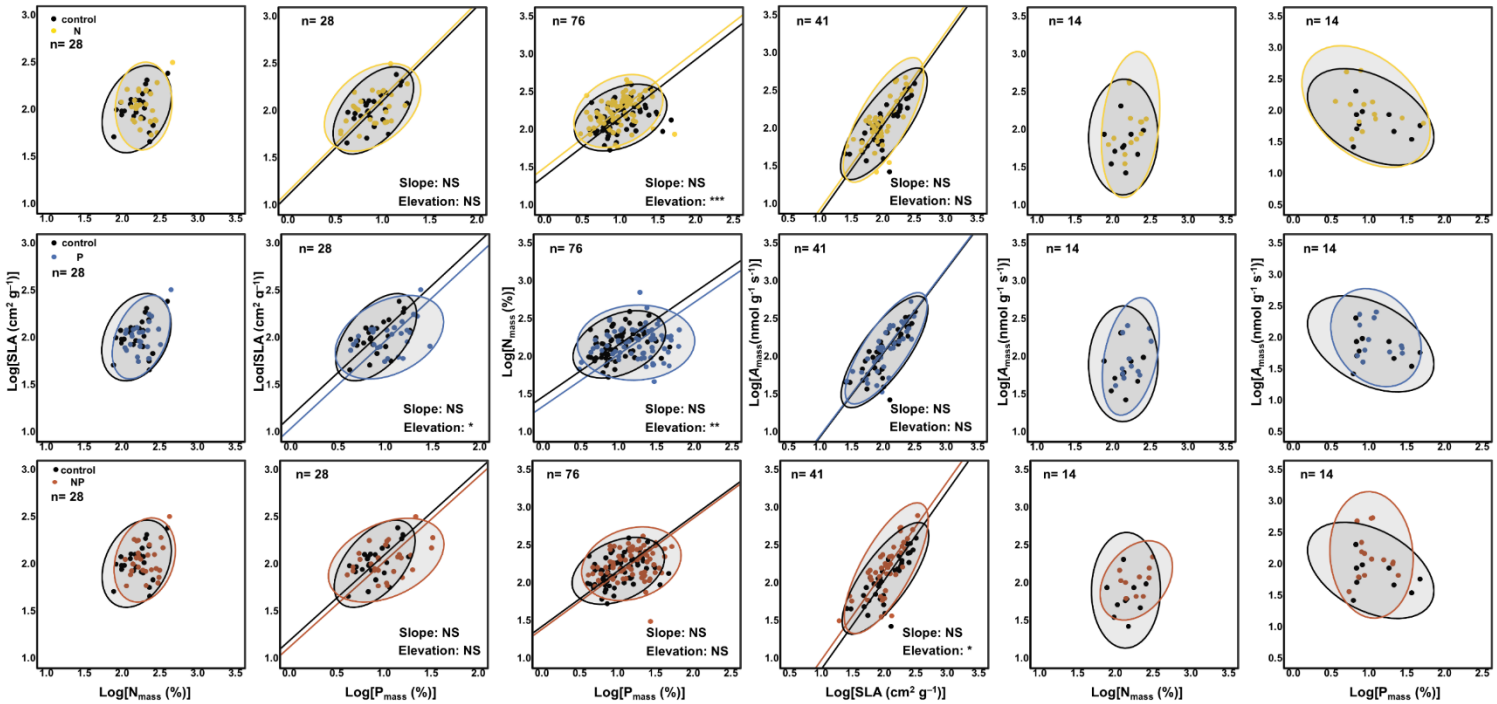

a
N
P
NP
C3
C4
Changes in percentage (%)
SLA
Amass
Nmass
Pmass
PNUE
PPUE


b
Monocotyledon
Dicotyledon
Changes in percentage (%)
SLA
Amass
Nmass
Pmass
PNUE
PPUE

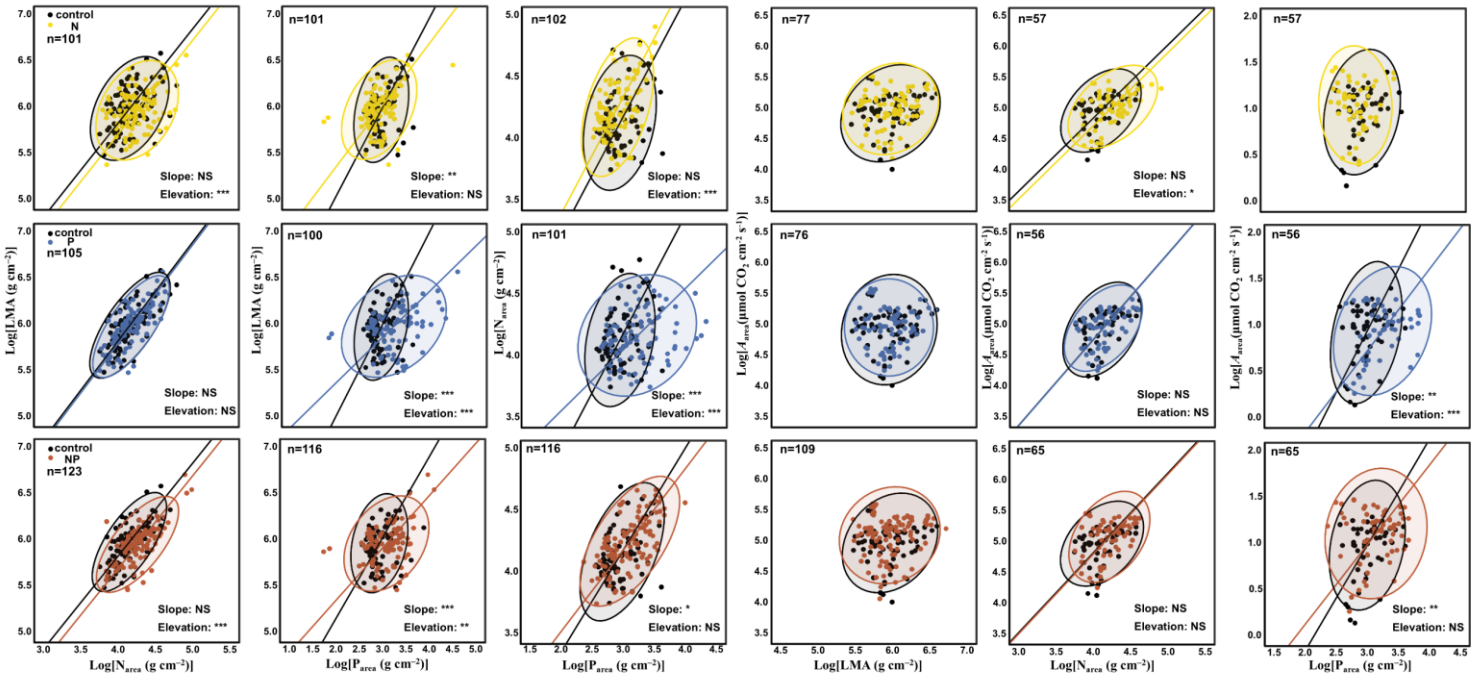


Supplementary Fig. 5 The mean effect sizes of nitrogen (N) and phosphorus (P) addition on plant functional traits across different categories. a, C_3 versus C_4

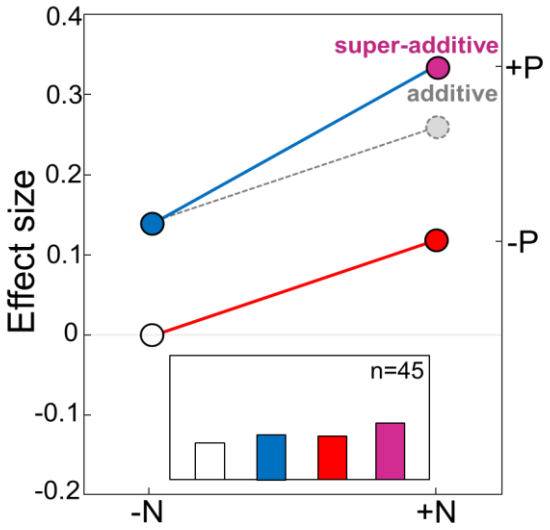
photosynthetic pathway species. **b**, Monocotyledon versus dicotyledon species. **c**, Herbaceous versus woody species. **d**, Field versus controlled-environment experiments. Error bars show the 95% credible intervals. Paired *t*-tests were used to compare the responses of plant functional groups between the control and the N and P addition treatments. The significance of effect sizes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. SLA, specific leaf area; A_{mass}, mass-based net photosynthetic rate; N_{mass}, mass-based nitrogen concentration; P_{mass}, mass-based phosphorus concentration; PNUE, photosynthetic N-use efficiency; PPUE, photosynthetic P-use efficiency.



Supplementary Fig. 6 Response of leaf trait relationships to nutrient additions under NP co-limitation. Black and colored ellipses denote the 95% confidence regions for control and nutrient-addition treatments, respectively. Bold lines represent Standardized Major Axis (SMA) regressions for each trait pair. Sample size for each group is indicated adjacent to its ellipse. Homogeneity of SMA slopes was assessed by a permutation test, and differences in SMA elevations were evaluated using the SMA equivalent of ANCOVA (see Supplementary Table S4 for full statistics). Trait combinations lacking a significant correlation under any treatment were omitted. Statistical significance is indicated: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.



Supplementary Fig. 7 Response of area-based leaf trait relationships to nutrient additions. Black and colored ellipses denote the 95% confidence regions for control and nutrient-addition treatments, respectively. Bold lines represent Standardized Major Axis (SMA) regressions for each trait pair. Sample size for each group is indicated adjacent to its ellipse. Homogeneity of SMA slopes was assessed by a permutation test, and differences in SMA elevations were evaluated using the SMA equivalent of ANCOVA (see Supplementary Table S5 for full statistics). Trait combinations lacking a significant correlation under any treatment were omitted. Statistical significance is indicated: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.



Supplementary Fig. 8 Photosynthetic responses to nutrient co-limitation. Interaction plots illustrate the responses to factorial addition of two nutrients by using log ratio effect size and relative responses, according to Harpole *et al*. (2011). The *Y*-axis represents log ratio effect size of response of photosynthesis (i.e., photosynthetic responses to nutrient addition relative to controls). Super-additive indicates that the combined effect of adding both nutrients leads to a greater increase in photosynthesis compared with the summed photosynthetic increase from single additions. Insert bar graphs illustrate the relative photosynthesis resulting from nutrient additions.

Supplementary Table 1 Results of Egger's regression test.

Leaf trait	Treatment	Egger's regression test	
		***Z*-value**	***P*-value**
SLA	N	1.77	>0.05
	P	1.20	>0.05
	NP	0.94	>0.05
N_{mass}	N	1.81	>0.05
	P	1.76	>0.05
	NP	0.37	>0.05
P_{mass}	N	1.70	>0.05
	P	0.36	>0.05
	NP	1.63	>0.05
A_{mass}	N	1.46	>0.05
	P	0.92	>0.05
	NP	1.87	>0.05

For each leaf trait and treatment (nitrogen (N), phosphorus (P), and NP), the table reports the Egger test statistic (Z = intercept/standard error) and two-tailed P-value. Values $|Z| < 1.96$ and two-tailed $P > 0.05$ indicate no publication bias. See Supplementary Fig. 2 for the corresponding funnel plots. SLA, specific leaf area; N_{mass}, mass-based nitrogen concentration; P_{mass}, mass-based phosphorus concentration; A_{mass}, mass-based net photosynthetic rate.

Supplementary Table 2 Classification of nutrient limitations.

Limitation	N limitation (RR_N >0.2)		no N limitation (RR_N < 0.2)	
	P limitation (RR_P >0.2)	no P response (RR_P< 0.2)	P limitation (RR_P >0.2)	no P response (RR_P < 0.2)
NP co-limitation (RR_{NP}>0.2)	NP co-limitation	N limitation	P limitation	NP co-limitation
no NP response (RR_{NP}<0.2)	NP co-limitation	unexpected	unexpected	No N-P response

Supplementary Table 3 Sample sizes for different patterns of nutrient limitation.

Nutrient limitation pattern	Total number of groups		
	Field experiments	Environmentally controlled experiments	Total
N limitation	12	1	13
P limitation	28	4	32
NP co-limitation	66	42	108
No N-P response	20	2	22
unexpected	23	1	24
Total Number of Groups	150	49	199

Supplementary Table 4 Results of standardized major axis regression (SMA) regression of mass-based leaf trait correlations under nitrogen (N), phosphorus (P) and combined NP additions. To assess homogeneity in SMA slopes, a permutation test was performed, while differences in SMA elevations were evaluated using the SMA analog of standard ANCOVA.

Trait pair (X and Y)	Treatment	n	Control				Group				Slope Homogeneity (*P*)	Shift in elevation (*P*)
			Slope 95% CI	Elevation 95% CI	R^2	*P*	Slope 95% CI	Elevation 95% CI	R^2	*P*		
N_{mass} and SLA	N	100	1.54(1.35, 1.75)	-0.21(-0.28, -0.14)	0.20	<0.001	1.54(1.35, 1.75)	-0.25(-0.32, -0.17)	0.21	<0.001	0.37	**<0.001**
	P	102	1.47(1.29, 1.66)	-0.19(-0.25, -0.12)	0.23	<0.001	1.47(1.29, 1.66)	-0.19(-0.26, -0.13)	0.21	<0.001	0.83	0.44
	NP	120	1.56(1.38, 1.76)	-0.23(-0.29, -0.16)	0.12	<0.001	1.56(1.38, 1.76)	-0.27(-0.34, -0.19)	0.03	0.06	0.86	**<0.001**
P_{mass} and SLA	N	91	0.41(0.35, 0.46)	0.31(0.30, 0.32)	0.18	<0.001	0.41(0.35, 0.46)	0.33(0.31, 0.34)	0.14	<0.001	0.17	**<0.05**
	P	96	0.45(0.37, 0.54)	0.31(0.30, 0.32)	0.18	<0.001	0.33(0.27, 0.41)	0.28(0.27, 0.29)	0.10	<0.05	**<0.05**	**<0.001**
	NP	113	0.42(0.37, 0.48)	0.30(0.29, 0.31)	010	<0.01	0.42(0.37, 0.48)	0.28(0.26, 0.29)	0.01	0.31	0.21	**<0.01**
P_{mass} and N_{mass}	N	203	0.29(0.27, 0.32)	0.33(0.33, 0.34)	0.13	<0.001	0.29(0.27, 0.32)	0.36(0.35, 0.36)	0.11	<0.001	0.16	**<0.001**
	P	197	0.30(0.27, 0.33)	0.34(0.33, 0.34)	0.15	<0.001	0.30(0.27, 0.33)	0.31(0.30, 0.32)	0.03	<0.05	0.11	**<0.001**
	NP	269	0.31(0.28, 0.34)	0.33(0.33, 0.34)	0.11	<0.001	0.31(0.28, 0.34)	0.31(0.28, 0.34)	0.14	<0.001	0.47	0.46
SLA and A_{mass}	N	76	1.41(1.23, 1.62)	-0.14(-0.21, -0.08)	0.23	<0.001	1.41(1.23, 1.62)	-0.13(-0.19, -0.06)	0.29	<0.001	0.76	0.22
	P	72	1.58(1.38, 1.82)	-0.19(-0.26, -0.12)	0.25	<0.001	1.58(1.38, 1.82)	-0.18(-0.25, -0.11)	0.37	<0.001	0.78	0.59
	NP	111	1.45(1.27, 1.64)	-0.14(-0.20, -0.09)	0.19	<0.001	1.45(1.27, 1.64)	-0.12(-0.18, -0.06)	0.31	<0.001	0.80	**<0.05**
N_{mass} and A_{mass}	N	57	2.18(1.86, 2.56)	-0.43(-0.54, -0.31)	0.28	<0.001	2.18(1.86, 2.56)	-0.48(-0.60, -0.35)	0.37	<0.001	0.47	**<0.001**
	P	62	2.16(1.83, 2.53)	-0.43(-0.54, -0.31)	0.20	<0.001	2.16(1.83, 2.53)	-0.43(-0.55, -0.31)	0.28	<0.001	0.59	0.81
	NP	67	2.03(1.73, 2.38)	-0.38(-0.49, -0.27)	0.20	<0.001	2.03(1.73, 2.38)	-0.41(-0.53, -0.29)	0.14	<0.05	0.96	**<0.05**
P_{mass} and A_{mass}	N	57	0.51(0.42, 0.61)	0.28(0.26, 0.30)	0.12	<0.05	0.60(0.47, 0.78)	0.32(0.30, 0.35)	0.05	0.09	**<0.05**	**<0.01**
	P	62	0.63(0.53, 0.75)	0.27(0.25, 0.29)	0.12	<0.01	0.63(0.53, 0.75)	0.22(0.20, 0.24)	0.21	<0.001	0.12	**<0.01**
	NP	67	0.66(0.56, 0.78)	0.28(0.26, 0.30)	0.10	<0.05	0.66(0.56, 0.78)	0.26(0.24, 0.29)	0.10	<0.05	0.67	0.24

Significant results (P<0.05) are shown in bold. SLA, specific leaf area; A_{mass}, mass-based net photosynthetic rate; N_{mass}, mass-based nitrogen concentration; P_{mass}, mass-based phosphorus concentration.

Supplementary Table 5 Results of standardized major axis (SMA) regression analyses of pairwise leaf-trait relationships in response to nutrient additions under co-limitation conditions. To assess homogeneity in SMA slopes, a permutation test was performed, while differences in SMA elevations were evaluated using the SMA analog of standard ANCOVA.

Trait pair (X and Y)	Treatment	n	Control				Group				Slope Homogeneity (*P*)	Shift in elevation (*P*)
			Slope 95% CI	Elevation 95% CI	R^2	*P*	Slope 95% CI	Elevation 95% CI	R^2	*P*		
SLA and N_{mass}	N	28	1.13(0.96, 1.33)	-0.03(-0.09, 0.03)	0.53	<0.001	1.13(0.96, 1.33)	-0.03(-0.09, 0.03)	0.53	<0.001	0.38	0.32
	P	28	1.08(0.92, 1.25)	-0.01(-0.07, 0.04)	0.53	<0.001	1.08(0.92, 1.25)	-0.01(-0.06, 0.04)	0.57	<0.001	0.85	0.71
	NP	28	1.11(0.95, 1.29)	-0.02(-0.08, 0.03)	0.53	<0.001	1.11(0.95, 1.29)	0.00(-0.05, 0.06)	0.50	<0.001	0.57	**<0.05**
SLA and P_{mass}	N	28	0.44(0.34, 0.56)	0.32(0.31, 0.34)	0.25	<0.01	0.44(0.34, 0.56)	0.33(0.32, 0.35)	0.17	<0.05	0.29	0.34
	P	28	0.43(0.34, 0.56)	0.32(0.31, 0.34)	0.25	<0.01	0.43(0.34, 0.56)	0.30(0.28, 0.32)	0.10	0.09	0.26	**<0.05**
	NP	28	0.42(0.33, 0.55)	0.33(0.31, 0.34)	0.25	<0.01	0.42(0.33, 0.55)	0.31(0.29, 0.33)	0.12	0.08	0.18	0.28
N_{mass} and P_{mass}	N	76	0.35(0.30, 0.40)	0.34(0.33, 0.35)	0.15	<0.001	0.35(0.30, 0.40)	0.36(0.35, 0.37)	0.11	<0.01	0.98	**<0.01**
	P	76	0.34(0.29, 0.39	0.34(0.33, 0.35)	0.15	<0.001	0.34(0.29, 0.39)	0.31(0.30, 0.33)	0.01	0.44	0.54	**<0.01**
	NP	76	0.36(0.31, 0.42)	0.34(0.33, 0.35)	0.15	<0.001	0.36(0.31, 0.42)	0.33(0.32, 0.34)	0.05	0.06	0.63	0.35
SLA and A_{mass}	N	41	1.13(0.96, 1.33)	-0.03(-0.09, 0.03)	0.53	<0.001	1.13(0.96, 1.33)	-0.02(-0.08, 0.04)	0.47	<0.001	0.38	0.32
	P	41	1.08(0.92, 1.25)	-0.01(-0.07, 0.04)	0.53	<0.001	1.08(0.92, 1.25)	-0.01(-0.06, 0.04)	0.57	<0.001	0.85	0.71
	NP	41	1.11(0.95, 1.29)	-0.02(-0.08, 0.03)	0.53	<0.001	1.11(0.95, 1.29)	0.00(-0.05, 0.06)	0.50	<0.001	0.57	**<0.05**
N_{mass} and A_{mass}	N	10	2.23(1.47, 3.36)	-0.46(-0.78, -0.13)	<0.001	0.94	2.23(1.47, 3.36)	-0.47(-0.81, -0.12)	0.08	0.34	0.45	0.79
	P	10	2.11(1.41, 3.14)	-0.42(-0.72, -0.11)	<0.001	0.94	2.11(1.41, 3.14)	-0.44(-0.75, -0.12)	0.13	0.20	0.62	0.54
	NP	10	2.25(1.48, 3.42)	-0.46(-0.80, -0.13)	<0.001	0.94	2.25(1.48, 3.42)	-0.46(-0.82, -0.11)	<0.001	0.96	0.41	0.99
P_{mass} and A_{mass}	N	10	-0.56(-0.82, -0.38)	0.27(0.23, 0.32)	0.16	0.16	-0.56(-0.82, -0.38)	0.29(0.24, 0.34)	0.15	0.17	0.99	0.55
	P	10	-0.63(-0.94, -0.43)	0.27(0.23, 0.32)	0.16	0.16	-0.63(-0.94, -0.43)	0.32(0.27, 0.36)	0.03	0.53	0.51	0.12
	NP	10	-0.69(-1.04, -0.46)	0.27(0.22, 0.32)	0.16	0.16	-0.69(-1.04, -0.46)	0.33(0.28, 0.39)	<0.001	0.93	0.25	0.08

Significant results (*P*<0.05) are shown in bold. SLA, specific leaf area; A_{mass}, mass-based net photosynthetic rate; N_{mass}, mass-based nitrogen concentration; P_{mass}, mass-based phosphorus concentration.

Supplementary Table 6 Results of standardized major axis regression (SMA) regression of area-based leaf trait correlations under nitrogen (N), phosphorus (P) and combined NP additions. To assess homogeneity in SMA slopes, a permutation test was performed, while differences in SMA elevations were evaluated using the SMA analog of standard ANCOVA.

Trait pair (X and Y)	Treatment	n	Control				Group				Slope Homogeneity (*P*)	Shift in elevation (*P*)
			Slope 95% CI	Elevation 95% CI	R^2	*P*	Slope 95% CI	Elevation 95% CI	R^2	*P*		
LMA and A_{area}	N	77	1.44(1.23, 1.69)	-0.43(-0.61, -0.25)	0.05	0..06	1.44(1.23, 1.69)	-0.42(-0.60, -0.24)	0.02	0.20	0.71	0.30
	P	76	1.67(1.42, 1.96)	-0.60(-0.81, -0.39)	0.01	0.42	1.67(1.42, 1.96)	-0.59(-0.81, -0.39)	<0.001	0.98	0.59	0.58
	NP	109	1.50(1.32, 1.71)	-0.47(-0.62, -0.31)	0.06	<0.05	1.50(1.32, 1.71)	-0.46(-0.61, -0.30)	0.01	0.24	0.41	**<0.05**
LMA and P_{area}	N	101	0.38(0.33, 0.44)	0.60(0.57, 0.62)	0.08	<0.01	0.38(0.33, 0.44)	0.60(0.58, 0.63)	0.13	<0.001	**<0.01**	0.24
	P	100	0.35(0.30, 0.41)	0.61(0.59, 0.64)	0.09	<0.01	0.35(0.30, 0.41)	0.60(0.58, 0.63)	0.09	<0.01	**<0.001**	**<0.001**
	NP	116	0.34(0.30, 0.40)	0.62(0.59, 0.64)	0.07	<0.05	0.34(0.30, 0.40)	0.61(0.59, 0.63)	0.08	<0.01	**<0.001**	**<0.01**
LMA and N_{area}	N	101	0.72(0.64, 0.82)	0.33(0.27, 0.39)	0.17	<0.001	0.72(0.64, 0.82)	0.32(0.26, 0.38)	0.10	<0.01	0.59	**<0.001**
	P	105	0.75(0.68, 0.83)	0.31(0.27, 0.36)	0.52	<0.001	0.75(0.68, 0.83)	0.31(0.27, 0.36)	0.53	<0.001	0.92	0.23
	NP	123	0.72(0.65, 0.79)	0.34(0.29, 0.38)	0.48	<0.001	0.72(0.65, 0.79)	0.33(0.28, 0.37)	0.46	<0.001	0.29	**<0.001**
N_{area} and P_{area}	N	102	0.66(0.58, 0.76)	0.31(0.27, 0.35)	0.08	<0.01	0,66(0.58, 0.76)	0.32(0.28, 0.37)	0.19	<0.001	0.99	**<0.001**
	P	101	0.51(0.45, 0.59)	0.38(0.34, 0.41)	0.08	<0.01	0.51(0.45, 0.59)	0.36(0.32, 0.40)	0.06	<0.01	**<0.001**	**<0.001**
	NP	116	0.53(0.47, 0.59)	0.37(0.34, 0.40)	0.26	<0.001	0.53(0.47, 0.59)	0.37(0.34, 0.40)	0.37	<0.001	**<0.05**	0.96
N_{area} and A_{area}	N	57	1.03(0.87, 1.22)	0.07(-0.04, 0.18)	0.17	<0.01	1.03(0.87, 1.22)	0.06(-0.06, 0.17)	0.22	<0.001	0.51	**<0.05**
	P	56	1.21(1.02, 1.43)	-0.05(-0.18, 0.08)	0.15	<0.01	1.21(1.02, 1.43)	-0.05(-0.18, 0.08)	0.19	<0.001	0.54	0.98
	NP	65	1.11(0.95, 1.31)	0.01(-0.10, 0.13)	0.14	<0.01	1.11(0.95, 1.31)	0.01(-0.10, 0.13)	0.15	<0.01	0.64	0.67
P_{area} and A_{area}	N	57	4.18(3.45, 5.07)	-2.01(-2.40, -1.62)	0.05	0.10	4.18(3.45, 5.07)	-1.90(-2.28, -1.53)	<0.01	0.69	0.16	**<0.01**
	P	56	5.18(4.28, 6.28)	-2.52(-2.98, -2.07)	0.10	<0.05	5.18(4.28, 6.28)	-2.68(-3.18, -2.19)	0.11	<0.05	**<0.01**	**<0.001**
	NP	65	4.13(3.44, 4.96)	-2.00(-2.35, -1.65)	0.06	<0.05	4.13(3.44, 4.96)	-2.01(-2.38, -1.65)	0.03	0.21	**<0.01**	0.67

Significant results (P<0.05) are shown in bold. SLA, specific leaf area; A_{mass}, mass-based net photosynthetic rate; N_{mass}, mass-based nitrogen concentration; P_{mass}, mass-based phosphorus concentration.