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A threefold difference in plant growth response to nitrogen addition between the laboratory and field experiments

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Abstract. Quantifying the magnitude of plant response to nitrogen (N) addition is critical in improving our understanding of vegetation productivity in terrestrial ecosystems. Numerous studies under both the laboratory (hereafter lab) and field conditions have shown significant increases in plant growth by N addition. However, differences exist when we integrate or compare the results between N-addition experiments under the lab and field conditions. Here, we performed a meta-analysis on observations from 139 field and 127 lab experiments to identify their differences and similarities in the N response of plant growth. Overall, there was a threefold difference in the N effect on plant biomass between the lab (+63.1%) and field (+22.2%) experiments. The magnitude of the lab–field difference varied among plant categories and plant tissues. For example, the larger N effect in the lab than field conditions was about twofold for the herbaceous plant but fourfold for the woody species. Furthermore, the N-induced increase in biomass was allocated more to above-ground parts in the field but equally to above- and below-ground parts under the lab conditions. We further showed that these differences were jointly attributed to the differential abiotic (i.e., environmental condition and N application methods) and biotic (i.e., interspecific interaction, age, and functional types) factors under these two experimental conditions. These findings highlight more attention should be paid when the results from the lab experiments are translated to understand the N response of the natural plants.

Key words: biomass; field; laboratory; meta-analysis; nitrogen addition; plant growth.

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INTRODUCTION

Nitrogen (N), an essential element of plant components (chlorophyll, protein, enzymes, etc.), tightly involves in regulating plant metabolisms and activities (Novoa and Loomis 1981, Lawlor et al. 2001). Data from long-term investigations under natural N deposition and N manipulative experiments have demonstrated that the external N loading can stimulate plant growth and ecosystem productivity (Pregitzer et al. 2008, Xia and Wan 2008, Thomas et al. 2010). On the one hand, the increased leaf N concentration under N addition implies higher plant photosynthesis rate (Evans 1989). On the other hand, N supply could improve the competitive ability of plants for capturing other resources (e.g., light, water, or other nutrients) via the allocation of carbon resources (Olff et al. 1990, Song et al. 2010). Results from those experimental studies have dramatically improved our understanding of the roles of plants in sustaining terrestrial carbon

sequestration especially under the increasing atmospheric N deposition (Lu et al. 2011*b*, Schulte-Uebbing and De Vries 2017).

Currently, most insights of the plant responses to environmental changes are based on manipulative experiments, which are conducted in either lab or field condition. Lab experiments can control the environmental factors to closely investigate the influences of changes and mechanisms underlying the natural observational phenomena (Allison et al. 2009). Field experiments, as the bridge between laboratory and natural conditions, can quantify the functions of the changes in natural ecosystems. These two experimental approaches are both important for exploring the ecological responses of terrestrial plants to environmental changes (Nelson and Ehlers 1984, Gundersen et al. 1998, Feng et al. 2018). Moreover, observations in the lab conditions are usually designed to improve the insights in the field, when plant performances in natural ecosystems are unobtainable. However, there are unavoidable differences in abiotic and biotic factors between the lab and field experiments. For example, environmental conditions of higher temperature but lower light intensity have been observed in the lab relative to natural experiments (Max et al. 2012). The differential environments (e.g., light, water, temperature) account for unexpected differences in plant performance between the field and the lab condition in many studies. For example, Poorter et al. (2016) suggest that the higher temperature and lower light intensity might be the primary reason for the higher specific leaf area and N concentration in the plant under the lab experiments. A meta-analysis (Loydi et al. 2013) shows higher soil water availability in the lab experiments could dampen the positive effect of litter on the seedling emergency. However, some other studies (Lin et al. 2010) have reported no differences in plant biomass responses between the pot and field experiments. In addition, most field experiments are conducted in natural ecosystems with mixed species, whereas single species is primarily adopted in the lab experiments. Thus, it is necessary to synthesize the differences and similarities in plant performances between the lab and field experiments.

As the most universal limiting nutrient across various terrestrial ecosystems (Elser et al. 2007, Lebauer and Treseder 2008), N availability is regulated by N application methods (e.g., added dose, frequency, and the length of the treatment; Magill et al. 2000, Högberg et al. 2006), which consequently has impacts on plant growth. For example, within a 30-yr N addition experiments in boreal forests, tree stem volume responds positively to low N dose (3.4 and 6.8 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$) across all years, whereas the positive response declines and even disappears at high dose of 10.8 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ after 10-yr fertilization (Högberg et al. 2006). Environmental conditions, for example, temperature, water, light, and other nutrients, can indirectly regulate the effects of N availability on plant growth. For example, the stimulation of N addition on plant growth could be facilitated by increasing water availability (Harpole et al. 2007) or dampened by the deficiency in phosphorus (P; Elser et al. 2007). Considering the regulation of N application methods and experimental conditions on the responses of plant growth to N addition, they are hypothesized as important contributors in the different plant performances between the lab and field experiments. Furthermore, the ignorance of interspecific interactions in the lab experiments has influenced the N response of plant growth via many processes (Firbank and Watkinson 1990, Fridley 2003). We then hypothesize that the interspecific interactions also regulate the different plant responses to N addition between the lab and field experiments due to the known differences in plant growth with monoculture and mixture (Zanetti et al. 1996, Jucker et al. 2014). Here, a global dataset with 2965 observations of plant biomass from 297 N fertilization experiments was constructed. A meta-analysis was applied to explore the following three questions: (1) whether the difference of plant performances to N addition exists between the lab and field experiments; (2) how such difference varies with plant growth forms and plant parts; and (3) how the abiotic (N application methods and environmental variables) and biotic factors regulate the differences in plant responses between the lab and field experiments.

Materials and Methods

Data collection

Peer-reviewed journal articles, published before April 2017, were searched using Web of

Science with the following search term combinations: (nitrogen deposition or nitrogen addition or nitrogen application or nitrogen input or nutrient fertiliz* or nitrogen supply or nitrogen enrichment) and (plant biomass or productivity or production or mass or growth). Articles meeting the following criteria were included in our analysis: (1) Both control and treatment were included; (2) the responses of seed plant biomass to N treatment were provided at the species level, and the means, standard deviations (SD) or standard errors (SE), and sample sizes (n) of the control and the treatment were also provided; (3) responses of plant parts (e.g., whole, above-ground parts, below-ground parts, shoot, leaf, stem, root) were reported; and (4) species from cropland ecosystem were excluded from our analysis.

According to the above criteria, experimental approaches included growth chambers, greenhouses, open-top chambers, pots, gardens, and natural habitats. In this research, gardens and natural habitats were classified as "field conditions," where the study variables are manipulated in natural conditions. Growth chambers, greenhouses, open-top chambers, and pots were defined as "lab conditions," in which the disturbances of the other variables are minimized. As a result, a database of 258 individual studies (131 in the field, 119 in the lab, and 8 in both the lab and field) with single N addition was included in our meta-analysis (Fig. 1; Appendix S1: Note S1). In line with the above criteria, studies including N addition with additional treatments (e.g., warming) were also included in our synthesis. In these studies, the treatment without N addition (warming) was regarded as control and the combined effects (warming + N) as N treatment. Then, a database of 97 studies of N addition with additional treatments (warming, water addition, P fertilizer, and high light) in either the lab or the field conditions was also established (Appendix S1: Note S2). For each study, data of the mean, n, and SD or SE were extracted either directly from tables and texts or indirectly from figures using GetData Graph Digitizer 2.24 (http://getdata-graph-digitizer.com/).

Site information of latitude, longitude, and mean annual temperature (MAT) was extracted from literatures. For some lab experiments that not reporting the exact site location, we checked and replaced the site coordinates with authors' affiliation, as most experiments were conducted in their own labs. For the site that not reporting MAT, we extracted this information from the global climate database by site location (http://www.worldclim.org/). Species information (functional types,

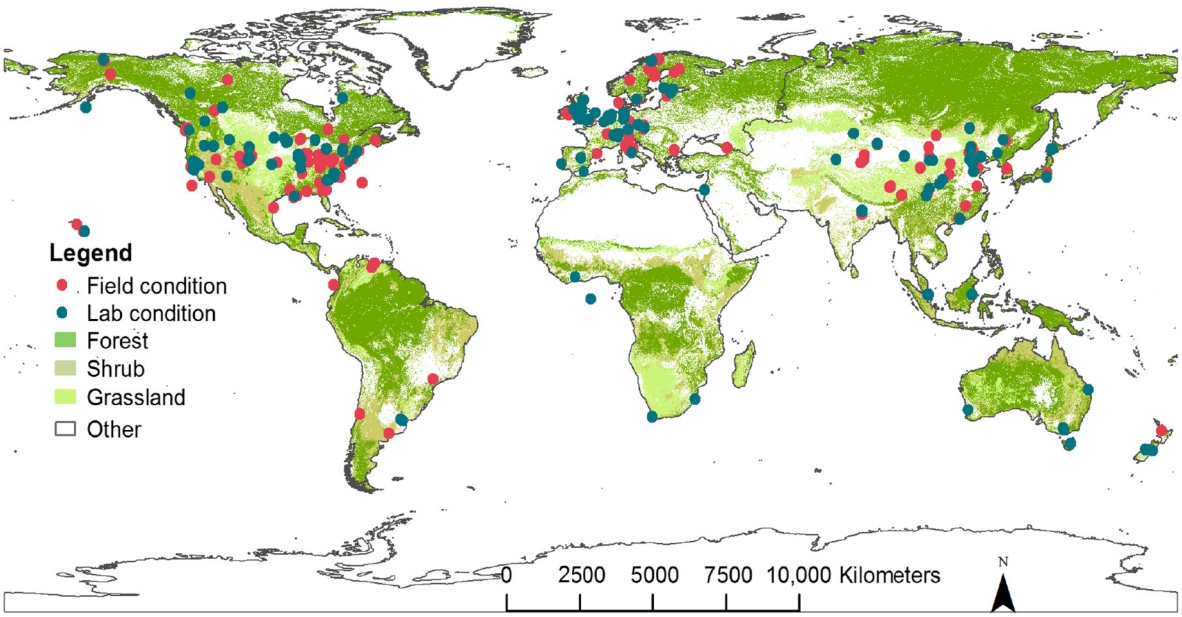

Fig. 1. The spatial distribution of N fertilization experiments in the lab and field condition.

interspecific interaction, and age) was also collected. Functional types of the species were checked referring to previous studies (Sage and Sultmanis 2016) and professional website (http://frps.iplant.cn/; https://pfaf.org/user/Default.aspx). Then, plant categories included growth forms (woody and herbaceous plant, or tree, shrub, grass, and forb), life history (annual and perennial herbs), photosynthesis way (C_3 and C_4 grass), and other plant functional groups (legume and non-legume, deciduous and evergreen trees, or broadleaved and coniferous trees). Meanwhile, variables related to the interspecific interaction (monoculture and mixture) were recorded. We defined "single species planted" as a monoculture condition and "species' number more than one" as a mixture condition in this study. We also collected age information for woody species, which was provided in most studies. Plant age ranged from <1 to 4 yr in the lab and to 100 yr in the field experiments and was divided into groups of <1, 1–10, 10–30, and >30 yr according to the age distribution.

In our database, N forms and application methods were also collected. N forms included ammonium nitrate (NH_4NO_3), urea, ammonium-N (NH_4^+-N), and nitrate-N (NO_3^--N). The N application methods included the dose (total external N amount per year), duration (the length of the experiment), and frequency (times of N application per year). In our database, the external doses ranged from <1.0 to 56.3 g $N{\cdot}m^{-2}$ yr^{-1} in the lab and to >100.0 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ in the field conditions. The duration in the lab and field conditions was 0.01–5.7 and 0.08–18.0 yr, respectively. The frequency was 1–1200 and 0.1–120 per year in the lab and field conditions, respectively. To test the functions of N treatment patterns on N-induced plant growth, the dose was grouped into <2.5, 2.5–5, 5–10, 10–20, 20–30, and >30 g $N{\cdot}m^{-2}{\cdot}yr^{-1}$, the duration into <0.5, 0.5–1, 1–2, 2–3, and >3 yr, and the frequency into <5, 5–10, 10–20, 20–30, 30–50, and >50 yr^{-1}. In addition, to assess how environmental variables regulate the N effect on plant growth in the lab and field, observations of N with additional treatments (Multi) were categorized into N with water addition (NW), P fertilization (NP), warming (NT), and high light (NHL).

Data analysis

The data were analyzed following the methods described by Hedges et al. (1999). The natural-logarithm-transformed response ratio (RR) was commonly used to evaluate the N effects on plant biomass for each observation as below:

$$\ln \mathrm{RR} = \ln \frac{X_\mathrm{t}}{X_\mathrm{c}} = \ln(X_\mathrm{t}) - \ln(X_\mathrm{c}) \tag{1}$$

where X_t and X_c are the means of the biomass in the treatment and control groups, respectively. Its variance (v) was estimated by:

$$v = \frac{S_t^2}{n_\mathrm{t} X_\mathrm{t}^2} + \frac{S_\mathrm{c}^2}{n_\mathrm{c} X_\mathrm{c}^2} \tag{2}$$

where n_t and n_c represent the sample size and S_t and S_c are the standard deviation of X_t and X_c, respectively. The reciprocal of its variance ($w = 1/v$) was considered as the weight of each lnRR. Then, the weighted response ratio (RR_{++}) was calculated in the random-effect model as below (m is the number of groups, and k is the number of comparisons):

$$\mathrm{RR}_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{K} w_{ij} \ln \mathrm{RR}_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{K} w_{ij}} \tag{3}$$

and its standard error was calculated as

$$S(\mathrm{RR}_{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m}\sum_{j=1}^{K} w_{ij}}} \tag{4}$$

Then, the 95% confidence interval (95% CI) was $\mathrm{RR}_{++} \pm 1.96\, S(\mathrm{RR}_{++})$ and was generated by bootstrapping the data using Metawin 2.0. The percentage change of a variable was back-transformed as

$$A = \left[\exp(\mathrm{RR}_{++}) - 1\right] \times 100\% \tag{5}$$

The effect of N addition on plant biomass was evaluated as significant if the 95% CI did not overlap zero. This meta-analysis also followed the theory of heterogeneity described by Gurevitch and Hedges (1993), in which total heterogeneity (Q_T) is divided into within-group (Q_w) and between-group (Q_b) heterogeneity. If Q_b is larger than a critical value, there would be a significant difference among different levels. Statistical significance was tested at the $P < 0.05$ level.

To evaluate to what extent plant response to N addition in the lab differed from that in the field conditions, we defined parameter R as the ratio of biomass percentage change in the lab relative to that in the field conditions, which was expressed as:

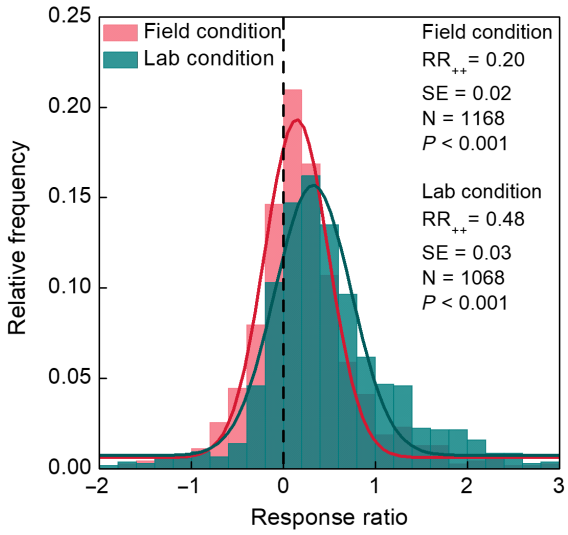

Fig. 2. The frequency distribution of the natural logarithm-transformed response ratios (lnRR) of the lab- (green) and field-plant (red) growth to N addition. The solid curves are Gaussian distribution fitted to the frequency data in the lab (green) and field (red) conditions, respectively. The *x*-axis is the lnRR, and the *y*-axis is relative frequency. The vertical dashed line is at lnRR = 0.

$$R = \frac{A_{\text{lab}}}{A_{\text{field}}} \quad (6)$$

where A_{lab} and A_{field} are percentage changes of plant biomass in the lab and field conditions, respectively. Only when A_{lab} and A_{field} were changed in the same direction, the method of *R*-value was used.

In addition, we used OriginPro 8.5 software to fitting relationships between the responses of plant biomass and the N application dose, duration, and frequency in the lab and field experiments.

Results

Higher estimation of plant growth to N addition in the lab relative to that in the field

N addition stimulated plant biomass in both the lab (63.1%) and field conditions (22.2%) across terrestrial seed plants in this analysis, with an *R*-value (the ratio of biomass percentage change in the lab relative to that in the field condition) of 2.8 ($P < 0.001$; Fig. 2, Table 1). Plant responses to N addition and their difference between the lab and field conditions depended

Table 1. Weighted biomass response ratio (RR++ ± 95% confidence interval) under N addition and their differences (*R*) between the lab and field experiments for all plant categories (values in the parentheses are sample size).

Categories	RR++ (sample size)		*R*	Field vs. Lab	
	Field	Laboratory		Q_b	*P*
Seed plant	0.20 ± 0.02 (1168)	0.48 ± 0.03 (1068)	2.8	177.5	< 0.001
Woody	0.12 ± 0.04 (514)	0.48 ± 0.04 (426)	4.5	139.0	< 0.001
Herbaceous	0.25 ± 0.03 (654)	0.49 ± 0.04 (642)	2.1	61.6	< 0.001
Tree	0.16 ± 0.05 (251)	0.44 ± 0.05 (348)	3.0	62.3	< 0.001
Shrub	0.08 ± 0.06 (263)	0.68 ± 0.11 (78)	11.0	106.5	< 0.001
Grass	0.29 ± 0.04 (423)	0.52 ± 0.04 (410)	1.9	35.3	< 0.001
Forb	0.18 ± 0.06 (231)	0.42 ± 0.06 (228)	2.6	21.9	< 0.001
Broadleaved tree	0.22 ± 0.07 (145)	0.51 ± 0.05 (223)	2.6	36.9	< 0.001
Coniferous tree	0.09 ± 0.06 (111)	0.31 ± 0.05 (125)	3.8	25.0	< 0.001
Evergreen tree	0.16 ± 0.06 (151)	0.43 ± 0.07 (127)	2.9	28.8	< 0.001
Deciduous tree	0.16 ± 0.08 (105)	0.45 ± 0.05 (221)	3.2	31.3	< 0.001
Annual herb	0.38 ± 0.08 (127)	0.64 ± 0.07 (163)	1.9	19.1	< 0.01
Perennial herb	0.22 ± 0.04 (527)	0.43 ± 0.04 (479)	2.1	37.1	< 0.001
C_3 grass	0.27 ± 0.07 (216)	0.54 ± 0.05 (314)	2.3	33.4	< 0.001
C4 grass	0.32 ± 0.07 (207)	0.44 ± 0.10 (96)	1.4	4.3	< 0.05
Non-legume	0.25 ± 0.08 (171)	0.44 ± 0.07 (214)	1.8	6.7	<0.05
Legume	0.01 ± 0.13 (60)	0.19 ± 0.29 (14)	10.4	8.1	< 0.05

Note: Between-group heterogeneity (Q_b) and probability (*P*) of nitrogen effects on biomass between lab and field experiments within different plant categories.

on growth forms (Fig. 3). For woody species, plant biomass was increased by 62.7% in the lab but only 13.8% in the field, with an *R*-value of 4.5 ($P < 0.001$; Table 1). Though greater stimulation of N on herbaceous biomass could be found in both the lab (63.5%) and field (29.2%) comparing with woody species, the difference ($R = 2.1$) was reduced. It may be attributed to the fewer differences on the growth of grasses ($R = 1.9$) and forbs ($R = 2.6$), than those of trees ($R = 3.0$) and shrubs ($R = 11.0$).

The differential responses of plant biomass to N addition between the lab and field experiments also varied on plant functional types (Fig. 3, Table 1). For tree species, the *R*-value ranged from 2.6 in the broadleaved trees to 3.8 in the coniferous trees. However, for the herbaceous species, similar differences were found between annual ($R = 1.9$) and perennial herbs ($R = 2.1$). The R-value of C_4 (1.4) was $<C_3$ (2.3) grass, because of higher stimulation for C_4 (38.9%) than for C_3 (31.0%) in the field, but lower stimulation for C_4 (56.8%) than for C_3 (72.0%) in the lab conditions. For the forbs, N addition had no effects on leguminous plant growth, but significant difference in the responses of non-leguminous plant

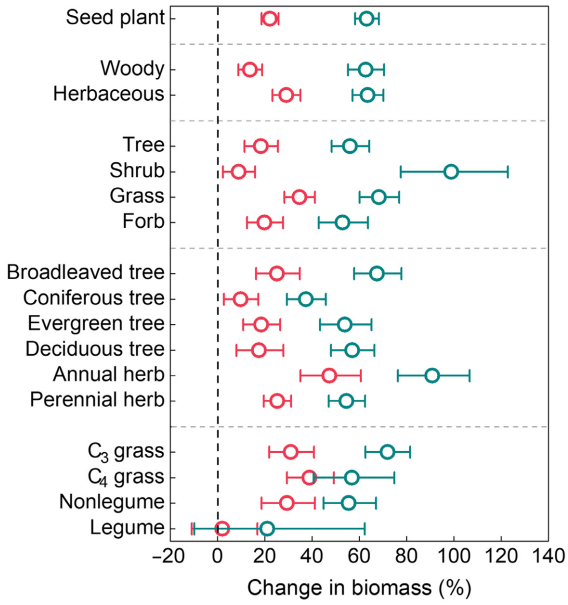

Fig. 3. The percentage change in biomass for plant functional types to N addition in the lab (green) and field condition (red). Values are mean ± 95% confidence intervals (CI). The vertical dashed line is at RR = 0.

growth was found between the lab and field experiments ($R = 1.8$).

Differential growth responses of plant parts to N addition between the lab and field conditions

N addition stimulated greater increases in above- and below-ground biomass under the lab conditions across all seed plants. When dividing above-ground parts into organs of leaf and stem, the stimulated increase in leaf biomass was equal in the lab (+36.2%) and field conditions (+33.2%), but a greater increase was shown on stem biomass in the lab (+39.4%) than field conditions (+9.7%, $R = 4.1$; Fig. 4b; Appendix S1: Table S1). In addition, the ratios in the response of above- relative to below-ground biomass (ΔAGB/ΔBGB) varied with experimental conditions (Fig. 4a). In the lab, due to the equal stimulations of N on above- (+61.7%) and below-ground growth (+56.1%), ΔAGB/ΔBGB ratio was close to 1.0. However, ΔAGB/ΔBGB ratio (4.4) was significantly higher in the field experiments ($P < 0.001$; Table 2), because of a higher response in above-ground biomass (+30.8%), but no change in below-ground biomass (+6.9%).

The magnitude of ΔAGB/ΔBGB ratio varied with growth forms. Higher responses in above-ground biomass were observed across various plant functional types for field-grown plants. However, the ΔAGB/ΔBGB in the lab condition for woody plants was 1.5, with a greater response in above- (+71.5%) than below-ground biomass (+45.0%; Table 2). It could be attributed to higher ΔAGB/ΔBGB ratio in trees (1.7) than shrubs (0.9). However, the ΔAGB/ΔBGB ratio for herbaceous plants was <1, with non-significant differences in responses between below- and above-ground parts both for grass (ΔAGB/ΔBGB = 0.6) and for forb (ΔAGB/ΔBGB = 1.1; Table 2).

The additional influence of other treatments

N effects on plant biomass were affected by additional factors (water, NW; phosphorus, NP; temperature, NT; and high light, NHL; Fig. 5). Significant stimulation of plant biomass was observed in both the lab (88.5%; $P < 0.05$) and field conditions (27.2%; $P < 0.05$) under N with additional treatments comparing with N alone, with *R*-value of 3.3 (Table 3). In addition, N effects on plant biomass in the lab and field

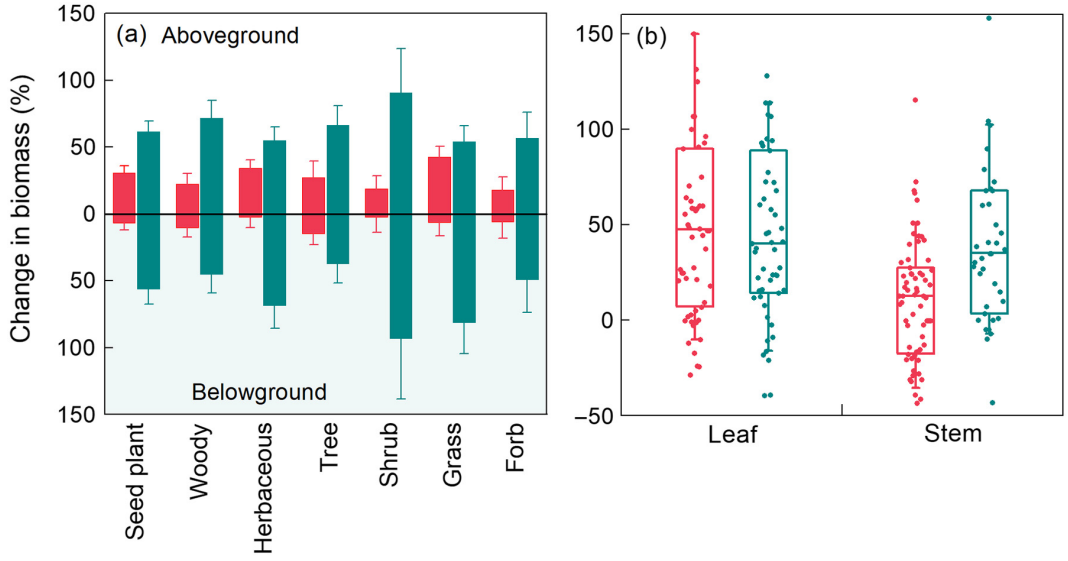

Fig. 4. Responses of above- and below-ground biomass for plant functional types (a), and the responses of leaf and stem growth of terrestrial seed plants (b) under N addition in the lab (green) and field condition (red), respectively. Box–scatterplot in panel b shows the data distribution by integrating box with actual data against the same scale. The box indicates the 25th and 75th percentile, and the whiskers show the 10th and 90th percentile.

Table 2. Weighted biomass response ratio (RR++) of above- and below-ground part under N addition and the ratio of above- to below-ground biomass percentage change (ΔAGB/ΔBGB) across plant categories under the lab and field conditions, respectively (values in the parentheses are sample sizes).

Categories	RR++ (sample size)		ΔAGB/ΔBGB	AGB vs. BGB	
	AGB	BGB		Q_b	*P*
Field					
Seed plant	0.26 ± 0.04 (531)	0.06 ± 0.04 (341)	4.4	41.0	< 0.001
Woody	0.20 ± 0.06 (174)	0.09 ± 0.06 (209)	2.1	7.5	< 0.01
Herbaceous	0.29 ± 0.04 (357)	0.02 ± 0.08 (132)	16.0	28.1	< 0.001
Tree	0.24 ± 0.09 (63)	0.13 ± 0.07 (156)	1.8	4.8	< 0.05
Shrub	0.17 ± 0.08 (111)	−0.02 ± 0.12 (53)	8.6	10.2	< 0.01
Grass	0.35 ± 0.05 (238)	0.06 ± 0.09 (92)	6.6	18.4	< 0.01
Forb	0.16 ± 0.07 (119)	−0.06 ± 0.14 (40)	3.0	9	< 0.05
Lab					
Seed plant	0.48 ± 0.04 (543)	0.44 ± 0.07 (212)	1.0	0.6	0.54
Woody	0.53 ± 0.07 (213)	0.37 ± 0.10 (107)	1.5	9.1	< 0.05
Herbaceous	0.43 ± 0.06 (330)	0.52 ± 0.10 (105)	0.7	1.6	0.28
Tree	0.50 ± 0.08 (163)	0.31 ± 0.11 (88)	1.7	11.8	< 0.01
Shrub	0.64 ± 0.15 (50)	0.65 ± 0.26 (19)	0.9	0.04	0.8
Grass	0.43 ± 0.07 (225)	0.59 ± 0.13 (70)	0.6	2.8	0.1
Forb	0.44 ± 0.11 (105)	0.39 ± 0.17 (35)	1.1	0.2	0.7

Note: Between-group heterogeneity (Q_b) and probability (*P*) of N effect on above- and below-ground growth within different plant categories between the lab and field experiments.

conditions and their differences changed with additional treatments. The *R*-value under NW decreased to 0.9, due to a significant enhancement in the field (50.8%; $P < 0.001$) but a reduction in N effect in the lab (45.6%; $P < 0.05$) relative to N alone. However, the *R*-value was expanded up to 3.9 ($P < 0.001$) by NP fertilizer, which was attributed to a higher stimulation on

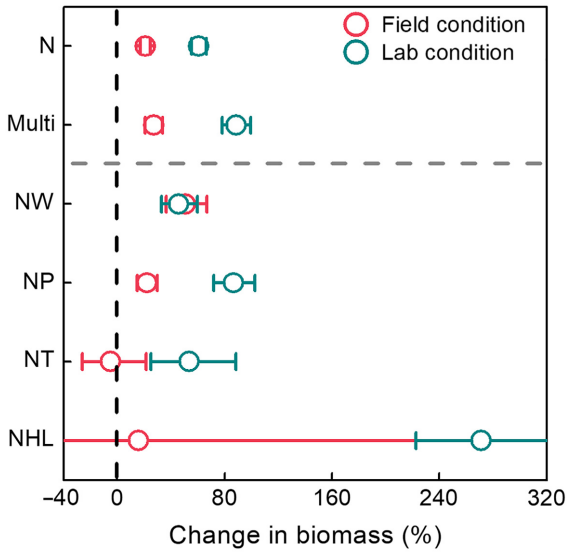

Fig. 5. Percentage change of plant biomass to only N addition (N), N with additional factors (Multi), N with water addition (NW), P fertilizer (NP), warming (NT), and high light (NL). Values are mean ± 95% confidence interval.

N effect in the lab (86.6%; $P < 0.001$) but no changes in the field (22.0%; $P > 0.1$). In addition, the greater response in the lab (271.0%) but no effect in the field (15.8%) also contributed to an enlarged *R*-value of 17.1 under NHL. There was no difference for plant response between NT and N in the lab ($P = 0.1$) and field conditions ($P > 0.1$).

The influence of N application variables on plant response to N addition

N application methods (the dose, the duration, and the frequency of the treatments) in the lab differed from those in the field experiments (Fig. 6). The dose was comparable between the lab (median value: 12.8 g $N \cdot m^{-2} \cdot yr^{-1}$) and the field conditions (13.1 g $N \cdot m^{-2} \cdot yr^{-1}$, $P > 0.1$). However, a significantly shorter experimental duration (median value: 0.7 yr) was found in the lab relative to that in the field (2.4 yr, $P < 0.001$), which was attributed to the higher addition frequency in the lab (median value: 56.9 yr^{-1} vs. 9.5 yr^{-1}). Our regression analysis revealed nonlinear relationships of N effects on plant biomass with the dose ($r^2 = 0.92$, $P < 0.01$) and the duration ($r^2 = 0.93$, $P < 0.001$) in the field experiments. The N saturation occurred when the dose was between 10 and 20 g $N \cdot m^{-2} \cdot yr^{-1}$ or when the duration was between 1 and 2 yr. However, the positive responses of plant biomass under N addition were linearly decreased with experimental duration ($r^2 = 0.93$, $P < 0.01$) in the lab conditions, while no clear relationship of plant response with N-applied dose was found across all the dose groups. Additionally, the relationship of N effects on plant biomass with the frequency was negative in the field ($r^2 = 0.72$, $P < 0.05$) but positive in the lab experiments ($r^2 = 0.42$, $P < 0.001$).

The data distributions of the N form were comparable between the lab and field experiments, both of which applied NH_4NO_3 most (Appendix S1: Fig. S1a). Meanwhile, greater responses of plant biomass in the lab than field experiments were observed across various N forms. However, the magnitude of differences was affected by the N forms, with the rank of NO_3^--N ($R = 6.0$), NH_4^+-N ($R = 4.2$), NH_4NO_3 ($R = 2.8$), and urea ($R = 1.5$; Appendix S1: Fig. S1b).

The role of species combination

Plants were grown mainly in the mixture condition (65.8%) in the field experiments but more in

Table 3. Between-group heterogeneity (Q_b) and probability (P) of N with additional factors relative to single N addition on plant growth under lab and field experimental conditions, and nitrogen effect on biomass between the lab and field experiments.

Categories	N vs. factors in field		N vs. factors in lab			Field vs. lab	
	Q_b	P	Q_b	P	R	Q_b	P
Multi	5.9	<0.05	4.2	<0.05	3.3	51.8	<0.001
NP	1.1	0.3	11.8	<0.001	3.9	46.8	<0.001
NW	16.2	<0.001	4.1	<0.05	0.9	0.3	0.60
NT	2.8	0.1	0	0.96	–	15.6	<0.001
NHL	0.0	0.82	139.0	<0.001	17.1	4.7	<0.01

Note: Abbreviations are N with water addition (NW), P fertilization (NP), warming (NT), and high light (NHL). The en dash means the R value is not calculated, for plant growth responds inversely to N addition between the field and lab condition.

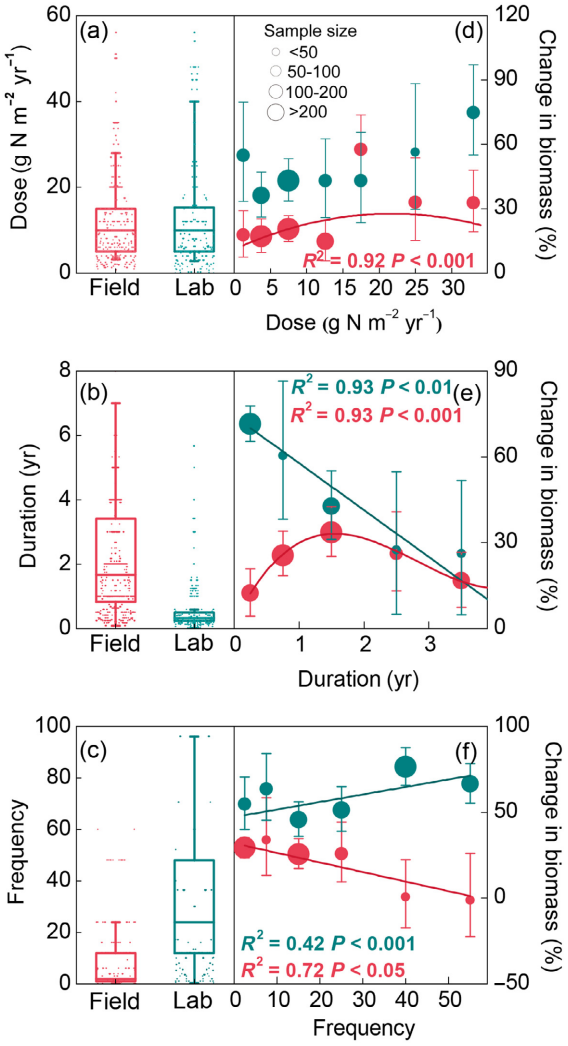

Fig. 6. The distribution of the external N dose, duration, and frequency of N fertilization experiment (a, b, c), and their effects on change of biomass (%, d, e, f), respectively, in the lab (green) and field condition (red). Box–scatterplot in panels a, b, and c shows the data distribution by integrating box with actual data against the same scale. The box indicates the 25th and 75th percentile, and the whiskers show the 10th and 90th percentile. Values in panels d, e, and f are mean ± 95% confidence interval.

monoculture (73.1%) under the lab conditions (Appendix S1: Fig. S2). Greater responses of plant biomass to N addition in the lab were found in both the mixture and monoculture conditions, with a higher *R*-value in the monoculture (2.9 vs. 1.9; Fig. 7; Table 4). For plants grown in the mixture, the N-induced increase in biomass was about twofold higher in the lab (41.0%) than field (21.3%) experiments. The difference in N response was enlarged under the monoculture, with 70.7% and 23.7% in the lab and field conditions, respectively.

The response differences of plant biomass to N addition between the lab and field conditions varied among growth forms in both the mixture and monoculture conditions. Larger differences were consistently observed among growth forms for plants grown in the monoculture, especially the woody species. However, no difference in plant responses between the lab and field experiments was detected for herbaceous species or shrub in the mixture.

Discussion

Overall, this meta-analysis indicates that N addition stimulates plant growth in both the lab (63.1%) and field (22.2%; Fig. 2) conditions. These findings are consistent with previous observations or meta-analysis of positive effects of N on the productivity or growth (Xia and Wan 2008, Thomas et al. 2010). Considering the different biotic and abiotic conditions that plants have experienced, Poorter et al. (2016) have pointed out the existence of the large differences in plant performance between the lab and field conditions. This study also finds a threefold difference in the response of plant biomass to N addition between the lab and field conditions across terrestrial seed plants. However, the difference varies with plant category and plant tissue. For example, the difference is doubled for woody species (about fourfold) comparing with that for herbs (about twofold). The N-induced biomass is allocated more to above-ground parts (+30.8%) in the field but equally to above- (+61.7%) and below-ground parts (+56.1%) in the lab conditions, which induces a larger difference in the below-ground response than that of above-ground parts.

Impacts of environmental variables on the different responses between the lab and field conditions

N addition has been widely reported to promote plant growth by improving soil N availability (Lu et al. 2011*a*). However, according to the Liebig's law of the minimum, many ecosystems once limited by N are now limited more by other

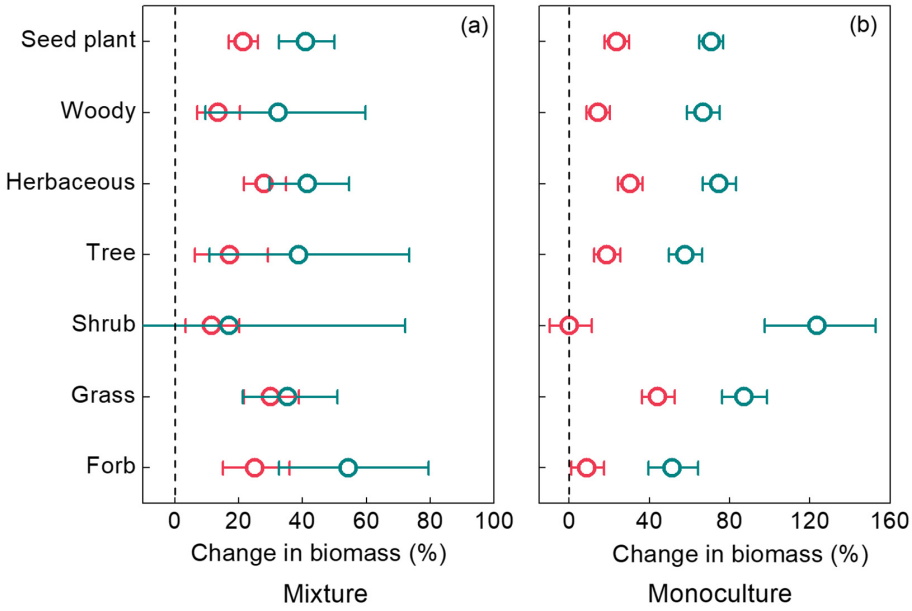

Fig. 7. Biomass responses of plant growth to N addition in the mixture (a) and monoculture (b) conditions under the lab (green) and field (red) across various plant functional types. Values are mean ± 95% confidence interval.

Table 4. The differences (R), between-group heterogeneity (Q_b), and probability (P) of N-induced plant growth in the mixture or monoculture condition between the lab and field within different plant categories.

	Mixture			Monoculture		
Category	R	Q_b	P	R	Q_b	P
Seed plant	1.9	12.2	< 0.001	2.9	133.9	< 0.001
Woody	2.3	5.4	<0.05	4.6	84.9	< 0.001
Herbaceous	1.4	3.4	0.06	2.4	53.4	< 0.001
Tree	2.2	4.0	<0.05	3.0	41.9	< 0.001
Shrub	1.4	0.1	0.71	587	39.5	< 0.001
Grass	1.1	0.6	0.41	1.9	26.5	< 0.001
Forb	2.1	2.6	0.11	5.7	35.5	< 0.001

resources under N addition. For example, the positive responses of plant biomass to N addition might be suppressed by the lack of water (Song et al. 2010, Wang et al. 2012) but enhanced by the additive P or light (Vitousek et al. 2010, Tripathi and Raghubanshi 2014). Therefore, the available water, light, and other nutrients, which together drive the differences of plant growth between the lab and field conditions (Poorter et al. 2016), might also be the main factors for the large differences of N stimulation on plant growth between the lab and field conditions.

By the synthetic analysis, our results show the dependence of plant response to N addition upon the environmental variables. The N-stimulated increases in plant biomass are enhanced by additive P or high light, but restrained by water addition in the lab conditions (Fig. 5), suggesting the P and light limitation under the external N. However, the significant positive effect of water on the N response in the field conditions emphasizes the water limitation on plant growth after N supply. Therefore, the difference of N effects between the lab and field conditions will be aggravated by P addition or high light in the lab, but efficiently reduced by water addition in the field conditions. Our results suggest that water availability might be the key factor to control the differential plant responses between the lab and field conditions.

The effects of N application methods on the response differences

N effects on plant growth can be directly affected by soil N availability. With N accumulated, plant growth is first promoted, then leveled off, and even declined when the supplied N exceeds plant N demands (i.e., N saturation;

Aber et al. 1989, Chen et al. 2016, Tian et al. 2016). Therefore, nonlinear relationships of plant growth with N dose or duration have been suggested by numerous studies (Magill et al. 2000, Högberg et al. 2006). However, negative even no significant relationships of plant growth with the changes in N dose or treatment duration have also been reported (Schulte-Uebbing and De Vries 2017, Wu et al. 2017). Moreover, the dependence of the plant response to N addition upon the N-addition frequency could be negative (Melgar et al. 2010), neutral (Zhang et al. 2015), or positive (Silber et al. 2003). These studies suggest that there might be interactive effects among N application methods on the plant response to N addition.

The dose of N addition (g $N \cdot m^{-2} \cdot yr^{-1}$) is comparable between the lab and field experiments in this study. However, the lab experiments have a shorter duration but with higher frequency than the field experiments (Fig. 6). The N effect on plant biomass in the field conditions firstly increases with the increasing N-applied dose and duration, and then reaches the peak when the added dose is about 10–20 g $N \cdot m^{-2} \cdot yr^{-1}$ or the duration is 1–2 yr. The N saturation in dose is comparable with previous observations (Bai et al. 2010, 10.5 g $N \cdot m^{-2} \cdot yr^{-1}$), but is greater than the synthesized value (5–6 g $N \cdot m^{-2} \cdot yr^{-1}$) in Tian et al. (2016). The N saturation in duration is shorter than the observation of Högberg et al. (2006) in a boreal forest (about 6 yr), but is consistent with Chen et al. (2016) in a tropical forest (1.5–3 yr). Contrary to the field observations, a negative linear relationship between N response and experimental duration is found under the lab conditions. The higher temperature (15.0 ± 0.24°C; Appendix S1: Fig. S3) and humidity in the lab experiments are likely to facilitate a lower N saturation threshold than that of medium temperature (especially at 8°C; Appendix S1: Fig. S3) and humidity in the field for the terrestrial ecosystems (Tian et al. 2016). Therefore, the greater response of the lab-grown plants to N addition with shorter time and higher frequency partly results in the response differences between lab- and field-grown plants. As the interactive effects of N application methods on the N effects, the implication of chronic experiments with low frequent N addition is essential for accurately investigating the natural plant response in the lab conditions.

The effects of interspecific interactions on N stimulation of plant growth

The greater N effects on plant biomass in the lab than field conditions are more evident when the plants are grown in monoculture than a mixture. This result indicates that interspecific interactions are also important in regulating the response differences between the lab and field experiments. The sufficient resource and low resource competition with other species could together support the high growth of plants in monoculture under the lab conditions. It suggests a potential N effect on plant growth with an average of 71.3%, which is larger than 29% in Lebauer and Treseder (2008). However, plants grown in the mixture are facing with complex interspecific interactions, which could reduce the differences in the plant responses to N addition between the lab and field conditions (Firbank and Watkinson 1990, Fridley 2003). Therefore, the lack of species interactions is an important factor leading to the higher N effects on plant biomass in the lab than field experiments.

Variations among plant tissues and plant functional types

In our study, N-stimulated increase in carbon resource is allocated more to above-ground parts in the field experiments, but equally to above- and below-ground parts in the lab (Fig. 4). As has been generalized by Bloom et al. (1985), biomass allocation is a strategy to promote adaptation and growth of plants via partitioning more biomass to restricted organs for capturing limited resources, for example, nutrients, light, and water. When the N limitation is alleviated, the resource competition would be shifted from below-ground for soil resources to above-ground for light resources in the field condition (Olff et al. 1990, Portsmuth and Niinemets 2006, Jung and Lal 2011). However, for the lab-grown plants, not only light but also other soil nutrients, like P, are limited under N addition (Fig. 5). The deficiencies of light and soil P stimulate carbon allocation equally to above- and below-ground growth in the lab (Jose et al. 2003, Song et al. 2010). In addition, greater stem response in the lab- than field-grown plants suggests stem growth is a light-capturing strategy for the plants in the lab conditions (Rogers and Siemann 2003, Portsmuth and Niinemets 2006). Our results highlight that environmental differences could be another reason for the differences in carbon

allocation between the lab- and field-grown plats under N addition.

The response differences of plant growth to N addition between the lab and field conditions also vary with plant functional types. Generally, the differences are greater for woody than herbaceous plants in both monoculture and mixture. This might be attributed to that the differences of plant age or size between the lab and field experiments are larger for woody than for herbaceous plants (Poorter et al. 2016). Woody plants are usually cultivated from seeds or seedlings in the lab, younger and smaller than field-grown plants. Numerous studies have demonstrated that the availability of both water and nutrient transportation is decreasing with woody age or size because of the increased hydraulic resistance (Bond 2000, Hubbard et al. 2001). As a consequence, a reducing rate of the leaf photosynthesis and an increasing carbon consumption with age are approved even under N fertilization (Ryan and Yoder 1997, Schulte-Uebbing and De Vries 2017, Wu et al. 2017). Comparing the age of the woody plants in the lab and field experiments, the mean age was much greater in the field (17.3 yr) than lab (0.8 yr) conditions (Fig. 8a). In addition, N addition significantly increased growth in seedlings (age < 1 yr) by 12.2%, but had no effect when age was more than 1 yr (Fig. 8b). Therefore, younger woody plants in the lab and the reduced plant responses to N addition with age are accounted for the greater differences for woody plants between the lab and field conditions (Vadeboncoeur 2010, Schulte-Uebbing and De Vries 2017, Wu et al. 2017). These results indicate that, including the abiotic conditions in the lab and field, biotic factors, for example, growth forms and age, should be additionally considered when translating the lab results to the field.

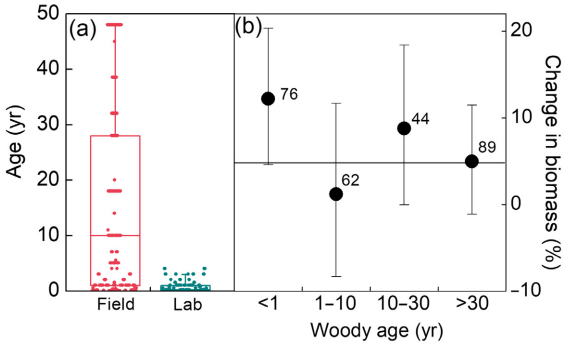

Fig. 8. The distribution of age in woody species in the lab (green) and field condition (red) (a), and the percentage change of biomass with age (mean ± 95% confidence interval) under N addition in the field (b). Box–scatterplot in panel a shows the data distribution by integrating box with actual data against the same scale. The box indicates the 25th and 75th percentile, and the whiskers show the 10th and 90th percentile. The value in panel b is the sample size.

Conclusions

Lab and field fertilization experiments are reliable approaches for exploring the ecological responses of terrestrial plants to N addition and quantifying N limitation on terrestrial carbon uptake. The transformation of insights from the lab experiments to the field conditions is particularly crucial for ecosystems in which plants are difficult to be measured or observed. However, both abiotic (i.e., environmental variables and N application methods) and biotic (i.e., growth forms, ages of species, interspecific interaction) factors contribute to the different responses of plant growth to N addition between the lab and field conditions. Such response differences between the lab and field experiments suggest the translation from the lab observations to patterns and processes in the field is a big challenge. However, our research highlights some adoptable pathways to integrate the findings from the two major experimental approaches. Thus, although the differences between lab and field experiments are unavoidable, insights in the lab experiments could increasingly improve the understandings of plant performance to environmental changes in the natural ecosystems.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2572/full