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Effects of human disturbance activities and environmental change factors on terrestrial nitrogen fixation

Running head: Human and environmental effects on N fixation

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ABSTRACT

Biological nitrogen (N) fixation plays an important role in terrestrial N cycling and represents a key driver of terrestrial net primary productivity (NPP). Despite the importance of N fixation in terrestrial ecosystems, our knowledge regarding the controls on terrestrial N fixation remains poor. Here, we conducted a meta-analysis (based on 852 observations from 158 studies) of N fixation across three types of ecosystems with different status of disturbance [no management, restoration (previously disturbed), and disturbance (currently disturbed)] and in response to multiple environmental change factors [warming, elevated carbon dioxide (CO_2), increased precipitation, increased drought, increased N deposition, and their combinations]. We explored the mechanisms underlying the changes in N fixation by examining the variations in soil physicochemical properties (bulk density, texture, moisture, and pH), plant and microbial characteristics (dominant plant species numbers, plant coverage, and soil microbial biomass), and soil resources [total carbon, total N, total phosphorus (P), inorganic N, and inorganic P]. Human disturbance inhibited non-symbiotic N fixation but not symbiotic N fixation. Terrestrial N fixation was stimulated by warming (+152.7%), elevated CO_2 (+19.6%), and increased precipitation (+73.1%) but inhibited by increased drought (-30.4%), N deposition (-31.0%), and combinations of available multiple environmental change factors (-14.5%), the extents of which varied among biomes and ecosystem compartments. Human disturbance reduced the N fixation responses to environmental change factors, which was associated with the changes in soil physicochemical properties (2–56%, $p<0.001$) and the declines in plant and microbial characteristics (3–49%, $p\leq0.003$) and soil resources (6–48%, $p\leq0.03$). Overall, our findings reveal for the first time the effects of multiple environmental change factors on terrestrial N fixation and indicate the role of human disturbance activities in inhibiting N fixation, which can improve our understanding, modeling, and prediction of terrestrial N budgets, NPP, and ecosystem feedbacks under global change scenarios.

Keywords: biological nitrogen fixation; environmental change; global change; human disturbance; nitrogen cycling; terrestrial ecosystems

INTRODUCTION

Biological nitrogen (N) fixation, a key process of N conversion [from dinitrogen gases (N_2) to biologically available N (NH_3)] performed by symbiotic or free-living N-fixing organisms, is an important source of N in Earth's ecosystems (Cleveland et al., 1999; Reed, Cleveland, & Townsend, 2011; Zehr, 2011). Because N is a nutrient whose availability constrains plant growth in terrestrial biomes (LeBauer & Treseder, 2008), biological N fixation plays a critical role in regional and global carbon (C) and N cycling (Kou-Giesbrecht & Menge, 2019; Levy-Varon et al., 2019; Wang, Houlton, & Field, 2007) and represents a driver of net primary productivity (NPP) (Dynarski & Houlton, 2018; Meyerholt, Zaehle, & Smith, 2016). Based on empirical data or modeling methods, many large-scale studies have indicated that biological N fixation can contribute 40–290 Tg N yr^{-1} to terrestrial ecosystems (Cleveland et al., 1999; Davies−Barnard & Friedlingstein, 2020; Galloway, Schlesinger, Levy, Michaels, & Schnoor, 1995; Galloway et al., 2004; Meyerholt et al., 2016; Vitousek, Menge, Reed, & Cleveland, 2013; Wang & Houlton, 2009). Large amounts of N inputs via N fixation alleviate N limitation in numerous biomes, e.g., forests (Moyes et al., 2016; Zackrisson, DeLuca, Nilsson, Sellstedt, & Berglund, 2004), grasslands (Reed et al., 2007b), croplands (Parvin et al., 2019), tundra (Rousk, Sorensen, & Michelsen, 2017c), and deserts (Su, Zhao, Li, Li, & Huang, 2011), and help to constrain concentrations of atmospheric carbon dioxide (CO_2; Meyerholt et al., 2016; Zehr, 2011). Despite the critical role of biological N fixation in terrestrial biomes, our knowledge regarding the controls on N fixation at the scale of terrestrial ecosystems remains very poor (Dynarski & Houlton, 2018; Zheng, Zhou, Luo, Zhao, & Mo, 2019; Reed et al., 2011), which has impeded our understanding, modeling, and prediction of global N budgets and NPP (Gruber & Galloway, 2008; Penuelas, Jannssens, Ciais, Obersteiner, & Sardans, 2020).

Human activities can act to stimulate or inhibit N fixation depending on context. For example, in some agricultural systems, tillage and irrigation can improve soil texture (Bronick & Lal, 2005) and thus increase biomass and N fixation rates of legume crops (Goh & Bruce, 2005; Wheatley, Macleod, & Jessop, 1995), whereas artificial fertilization (e.g., N-P-potassium) may reduce soil diazotrophic abundance and N fixation rates (Fan et al., 2019). In forest ecosystems, logging and burning may increase light availability and accelerate soil N losses, which favor N fixers (Stuiver, Gundale, Wardle, & Nilsson, 2015; Zackrisson et al., 2004). However, forest clear-cutting can not only reduce biomass of trees but also reduce biomass of epiphytic mosses (Palviainen, Finér, Mannerkoski, Piirainen, & Starr, 2005), and human harvest of forest floor may decrease availability of soil C and P, which constrains N fixation (Zheng et al., 2017). Although the above evidence indicates that human disturbance activities affect N fixation rates, it remains unclear whether human disturbance inhibits or stimulates N fixation rates at terrestrial biome scales.

Moreover, human-induced environmental change has potential effects on biological N fixation. For example, elevated N deposition increases soil N contents in natural ecosystems (e.g., tropical forests; Matson, McDowell, Townsend, & Vitousek, 1999), which inhibits the synthesis of nitrogenase, a class of enzymatic proteins responsible for N fixation, and reduces the competitive advantage of N fixers (Crews, 1999; Reed et al., 2011). Many simulated N-deposition studies have found declines in N fixation along ambient N-deposition gradients (Ackermann, Zackrisson, Rousk, Jones, & DeLuca, 2012; Leppänen, Salemaa, Smolander, Mäkipää, & Tiirola, 2013) and following experimental N-addition treatments (Barron et al., 2009; Cusack, Silver, & McDowell, 2009; Zheng et al., 2016a), although some studies have found a lack of N fixation response to N inputs (Jacot, Lüscher, Nösberger, & Hartwig, 2000; Reed, Cleveland, & Townsend, 2007a). Changes in precipitation patterns can affect N fixation because N fixers are sensitive to moisture variability (Reed et al., 2011). Many N-fixing microbes are anaerobes and high oxygen environments inhibit nitrogenase synthesis (Robson & Postgate, 1980). Increases in precipitation (or moisture) create low-oxygen conditions in saturated soils that favor N fixers (Reed et al., 2011; Su et al., 2011), whereas water deficit (or drought) often inhibits N fixation in forests (Gundale, Gustafsson, & Nilsson, 2009), deserts (Dickson, 2000), and greenhouses (Minucci, Miniat, Teskey, & Wurzburger, 2017). Global warming affects N fixation rates since nitrogenase functions at the optimal temperature of ~25^{o}C (Houlton, Wang, Vitousek, & Field, 2008). Previous studies have found positive effects of warming on N fixation (Gundale, Nilsson, Bansal, & Jäderlund, 2012a; Rousk, Pedersen, Dyrnum, & Michelsen, 2017b; Su et al., 2011), although some studies reported only minor effects (Bjerke, Zielke, & Solheim, 2003; Hutchins et al., 2007). However, extreme warming may induce water limitation on N fixers and thereby reduce N fixation rates (Gundale et al., 2012a; Gundale, Wardle, & Nilsson, 2012b). Elevated CO_2 may stimulate N fixation as it enhances growth of autotrophic N fixers (Lindo & Griffith, 2017), but this phenomenon has only been observed in agricultural and oceanic ecosystems (Guo et al., 2013; Hutchins, Fu, Webb, Walworth, & Tagliabue, 2013; Lam, Chen, Norton, & Armstrong, 2012). In several grasslands and forests, elevated CO_2 reduces the abundance of rhizobial N-fixing genes (Watanabe, Bowatte, & Newton, 2013) and induces nutrient (e.g., molybdenum and P) limitation on N fixation (Hungate et al., 2014). Thus, although multiple environmental change factors affect N fixation rates, the directions and magnitudes of the effects vary among biomes. To our knowledge, no published study has addressed how different environmental change factors (or their combinations) affect N fixation between different terrestrial biomes.

In this study, we aim to address how human disturbance activities and environmental change factors affect biological N fixation and explore the mechanisms underlying these effects. We compiled a global dataset of biological N fixation in different ecosystem compartments (soil, leaf litter, mosses, lichens, fresh leaves, root-nodules, and biological soil crusts) and in response to multiple environmental change factors (warming, elevated CO_2, increased precipitation, increased drought, increased N deposition, and their combinations). Our dataset covers a wide range of terrestrial ecosystems, including forests, grasslands, deserts, shrublands, heaths, tundra, wetlands, and croplands (Fig. S1). The ecosystems were divided into two categories: human-controlled greenhouses and field ecosystems. To investigate the effects of human disturbance on N fixation, we divided the field ecosystems into three types: (1) unmanaged ecosystems, (2) ecosystems in restoration, and (3) ecosystems under disturbance (see the Method section for details). To explore the mechanisms underlying the anthropogenic effects on N fixation under environmental change scenarios, we analyzed the changes in soil physicochemical properties (bulk density, texture, moisture, and pH), plant and microbial characteristics (dominant plant species numbers, plant coverage, and soil microbial biomass), and soil resources [C, N, P, nitrate (NO_3^-), ammonium (NH_4^+), and inorganic P] (data of soil physicochemical properties and resources cover bulk soils and sediments). We hypothesized that (1) human disturbance would inhibit terrestrial N fixation overall; (2) warming, elevated CO_2, and increased precipitation would stimulate N fixation but increased N deposition and drought would inhibit N fixation, and multiple environmental change factors combined would stimulate or inhibit terrestrial N fixation depending on the types of combined factors; and (3) human disturbance would inhibit the responses of N fixation to environmental change factors due to human-induced changes in soil physicochemical properties, plant and microbial biomass, and/or soil resources.

MATERIALS AND METHODS

Data sources

We systematically searched all peer-reviewed journal articles and theses using Google Scholar and Web of Science with the following keywords/phrases: ("nitrogen fixation" or "N fixation" or "N_2 fixation" or "dinitrogen fixation" or "nitrogenase") and ("warming" or "increasing temperature" or "elevated temperature" or “precipitation” or “rainfall” or “drought” or “carbon dioxide” or “CO_2” or “nitrogen deposition” or “N deposition” or “N input” or “nitrogen addition” or “N addition” or “nitrogen fertilization” or “N fertilization” or “global change”), and we further searched the reference lists of relevant articles. Each article was reviewed to determine whether it met the following criteria: (1) the effects of environmental change factors on N fixation could be isolated from other factors, e.g., P fertilization management; (2) N fixation rates could be directly extracted from the figures, tables, or texts; (3) N fixation rates were measured in at least one of the compartments, i.e., soil, leaf litter, mosses, lichens, fresh leaves, root-nodules, and biological soil crusts, and under at least one of the treatments, i.e., warming, elevated CO_2, increased precipitation, increased drought, increased N deposition, and their combinations (e.g., warming + increased precipitation); and (4) methods for measuring N fixation rates included acetylene reduction assay (ARA) or ^{15}N tracing methods (Zheng et al., 2019). Based on these standards, we obtained a meta-dataset of 852 observations (note that measurements at different sites, for different compartments, or under different treatments were considered to be different observations) from 158 literatures (published from April 1970 to January 2020; data source shown in Appendix) that investigated N fixation in terrestrial ecosystems (Fig. S1).

Data of geographical location (latitude and longitude), soil physicochemical properties (bulk density, clay + loam contents, moisture, and pH), plant and microbial characteristics [dominant plant species numbers, plant coverage, and soil microbial biomass carbon (MBC)], and soil resources (total C, total N, total P, NO_3^-, NH_4^+, and inorganic P) were collected directly from original publications or indirectly from their citations (data of soil physicochemical properties and resources cover bulk soils and sediments). We used Origin 9.1 (OriginLab Co., Northampton) digital plugin (Digitize) software to extract data from figures when the results were graphically reported. The studied ecosystems were divided into two categories (greenhouses and field ecosystems; Fig. S2−4). According to the status of human management/disturbance of ecosystem structure and functioning described by the articles or their citations (those studies or their citations without description of the history of the sites were excluded), we divided the field ecosystems into three types: (1) unmanaged ecosystems (which have not experienced human management or disturbance; e.g., unmanaged and pristine sites), (2) ecosystems in restoration [which have experienced human disturbance (e.g., cropping, burning, grazing, and cutting) in the past but are experiencing natural succession or under human protection currently; e.g., secondary sites (with regenerating vegetations from abandoned pastures and agricultural areas) and rehabilitated sites (under human management to promote restoration)]; (3) ecosystems under disturbance [which are experiencing intensive management or disturbance (e.g., cropping, harvesting, irrigation, grazing, and fertilization) currently; e.g., croplands and grazing areas].

Data analysis

Data were categorized into three ecosystem types (no management, restoration, and disturbance), nine biomes [forests (including tropical/subtropical, temperate, and boreal forests), grasslands (including swards, pastures, and steppes), deserts, shrublands, heaths, tundra, wetlands, croplands, and greenhouses (including glasshouse, chamber, and pot experiments that started with the incubation of seeds; those experiments that used samples collected from the field are excluded)], and seven ecosystem compartments [soil, leaf litter, mosses, lichens, fresh leaves, root-nodules (including samples collected from leguminous and actinorhizal trees, shrubs, herbs, and crops), and biological soil crusts]. We divided the pathways of N fixation into two groups: non-symbiotic N fixation (occurring in the soil, leaf litter, mosses, lichens, fresh leaves, and soil crusts) and symbiotic N fixation (occurring in the root-nodules; Reed et al., 2011). We compared N fixation rates (per unit mass and per unit area) in the control (no treatment) plots among three types of ecosystems (no management, restoration, and disturbance). We further conducted a meta-analysis to evaluate the responses of N fixation to multiple environmental change factors (warming, elevated CO_2, increased precipitation, increased drought, increased N deposition, and their combinations). The effect size of environmental change treatments for each observation was estimated by the natural logarithm transformed response ratio (lnRR):

$$\ln RR = \ln(\overline{X_t} / \overline{X_c}) = \ln(\overline{X_t}) - \ln(\overline{X_c}) \quad (1)$$

where $\overline{X_t}$ and $\overline{X_c}$ are the means of the treatments and controls, respectively. The variance (v) of response ratios was calculated as follows:

$$v = \frac{s_t^2}{n_t \overline{X_t^2}} + \frac{s_c^2}{n_c \overline{X_c^2}} \quad (2)$$

where n_t and n_c are the sample sizes of the variable in the treatments and controls, respectively, and s_t and s_c are the standard deviations (SDs) of the variable in the treatments and controls, respectively. If standard error (SE) was reported, we calculated the SD as follows:

$$SD = SE \times \sqrt{n} \quad (3)$$

where n is the sample size. If data were provided as means with a confidence interval (CI), we calculated the SD using the following equation:

$$SD = (CI_u - CI_l)\sqrt{n} / 2Z_{\alpha/2} \quad (4)$$

where CI_u and CI_l are the upper and lower limits of 95% CI, respectively, and $Z_{\alpha/2}$ is the Z score for a given level of significance (e.g., 1.96 when α=0.05). If SD, SE, or CI were not provided, we assigned SE as 1/4 of the means (Dynarski & Houlton, 2018). We used MetaWin 2.1 (Sinauer Associates Inc. Sunderland, MA, U.S.) software to calculate the weighted response ratio (RR_{++}) and 95% CI. Significant responses (p<0.05) were recognized if the 95% CI did not overlap with zero. The percentage changes for the variables following environmental change treatments were calculated as follows:

$$\text{Change}\ (\%) = \left[\exp(RR_{++}) - 1\right] \times 100\% \quad (5)$$

A one-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to examine the difference of N fixation rates, N fixation responses, soil physicochemical properties, plant and microbial characteristics, and soil resources among different ecosystem types. Logarithmic regression models were used to examine the relationships of response ratios against soil physicochemical properties, plant and microbial characteristics, and soil resources.

RESULTS

N fixation rates in different ecosystem types

In the control plots, N fixation rates differed among three types of ecosystems (i.e., unmanaged ecosystems, ecosystems in restoration, and ecosystems under disturbance; Fig. 1). Specifically, N fixation rates (per unit mass and per unit area) of the soil, leaf litter, mosses, and lichens were higher in unmanaged ecosystems than in the ecosystems in restoration [t_{21}=2.1, p=0.049 (per unit mass) and t_{24}=2.2, p=0.035 (per unit area)] or ecosystems under disturbance [$F_{2,61}$=5.2, p=0.008 (per unit mass) and $F_{2,23}$=3.7, p=0.041 (per unit area)]. In contrast, N fixation rates [expressed as percentage of N derived from atmosphere (%Ndfa)] of the root-nodules did not differ among three ecosystem types (p>0.05).

N fixation in response to environmental change factors

Different types of environmental change factors had different effects on N fixation (Fig. 2a, S5a). Warming, elevated CO_2, and increased precipitation enhanced N fixation rates [by 152.7% (95% CI: 146.7−158.8%), 19.6% (19.4−19.8%), and 73.1% (67.9–78.4%), respectively], whereas increased drought and N deposition reduced N fixation rates [by 30.4% (29.2–31.7%) and 31.0% (30.9–31.2%), respectively]. Treatments of 'warming + increased precipitation' and 'elevated CO_2 + warming' enhanced N fixation rates [by 144.0% (135.7–152.7%) and 63.2% (56.2−70.7%), respectively], whereas treatments of 'warming + increased drought', 'elevated CO_2 + increased drought', 'elevated CO_2 + increased N deposition', and 'warming + elevated CO_2 + increased drought' reduced N fixation rates [by 53.1% (49.2–56.7%), 10.7% (4.2–16.7%), 14.9% (14.7–15.1%), and 67.6% (57.4–75.3%), respectively]. Treatment of 'increased N deposition + increased precipitation' had no significant effect on N fixation rates. Overall, N fixation rates decreased [by 14.5% (14.3−14.7%)] following the treatment of combinations of available multiple (i.e., 2−3) environmental change factors.

Responses of N fixation to environmental change factors varied among biomes (Fig. 2b, S5b). Warming stimulated N fixation in (sub)tropical forests [by 17.6% (11.2–24.4%)], boreal forests [by 234.6% (198.7–274.8%)], grasslands [by 462.6% (431.9–495.1%)], deserts [by 373.3% (330.8–420.0%)], tundra [by 163.3% (142.3–186.2%)], heaths [by 170.7% (114.2–242.2%)], wetlands [by 205.9% (187.1–225.8%)], and greenhouses [by 62.4% (42.2–85.5%)] but did not affect N fixation in croplands. Elevated CO_2 stimulated N fixation in temperate forests [by 6.8% (0.3–13.8%)], grasslands [by 6.2% (2.1–10.5%)], deserts [by 173.2% (50.4–396.2%)], wetlands [by 57.4% (23.2–74.6%)], croplands [by 16.8% (11.0–22.9%)], and greenhouses [by 19.7% (19.5–19.9%)] but did not affect N fixation in (sub)tropical forests. Increased precipitation stimulated N fixation in (sub)tropical forests [by 83.2% (71.1–96.1%)], temperate forests [by 16.5% (4.2–30.2%)], boreal forests [by 169.8% (120.1–230.7%)], deserts [by 480.9% (423.7–544.4%)], tundra [by 22.0% (9.8–34.6%)], and wetlands [by 287.2% (237.6–344.0%)] but did not affect N fixation in grasslands. Increased drought stimulated N fixation in grasslands [by 27.9% (22.8–33.3%)] but inhibited N fixation in boreal forests [by 52.3% (35.0–65.0%)], deserts [by 44.0% (26.9–57.1%)], croplands [by 26.1% (22.2–29.8%)], and greenhouses [by

43.1% (41.8–44.4%)]. Increased N deposition inhibited N fixation in (sub)tropical forests [by 12.8% (10.1–15.5%)], temperate forests [by 43.3% (42.0–44.6%)], boreal forests [by 47.8% (38.9–55.3%)], grasslands [by 43.9% (40.6–47.0%)], shrublands [by 36.6% (22.2–52.3%)], croplands [by 40.0% (35.9–43.9%)], and greenhouses [by 31.0% (30.8–31.1%)] but did not affect N fixation in wetlands. Combinations of multiple environmental change factors stimulated N fixation in (sub)tropical forests [by 136.3% (127.8–145.1%)], tundra [by 204.3% (157.5–403.6%)], and croplands [by 31.3% (26.2–36.5%)] and inhibited N fixation in boreal forests [by 64.7% (58.8–69.8%)], grasslands [by 20.0% (13.8–25.8%)], and greenhouses [by 14.9% (14.7–15.1%)] but did not affect N fixation in shrublands.

Responses of N fixation to environmental change factors varied among ecosystem compartments (Fig. 2c, S5c). Warming stimulated N fixation in the soil/soil crusts [by 275.6% (262.3–289.4%)], leaf litter [by 290.5% (146.0–459.9%)], mosses [by 27.8% (22.0–34.0%)], lichens [by 206.3% (166.5–252.0%)], and root/root-nodules [by 158.6% (144.8–173.1%)]. Elevated CO_2 stimulated N fixation in the soil/soil crusts [by 5.1% (1.9–8.4%)], lichens [by 143.1% (53.5–284.8%)], root/root-nodules [by 19.6% (19.4–19.8%)] but did not affect N fixation in the mosses. Increased precipitation stimulated N fixation in the soil/soil crusts [by 107.6% (94.5–121.7%)], leaf litter [by 25.8% (11.9–41.4%)], mosses [by 120.1% (108.4–132.4%)], lichens [by 203.0% (150.8–266.1%)], and root/root-nodules [by 24.8% (16.5–33.7%)]. Increased drought inhibited N fixation in the soil/soil crusts [by 3.7% (0.6–7.8%)], mosses [by 52.3% (35.0–65.0%)], and root/root-nodules [by 35.1% (33.8–36.4%)]. Increased N deposition inhibited N fixation in the soil/soil crusts [by 45.8% (44.5–47.0%)], leaf litter [by 24.6% (19.2–29.6%)], mosses [by 11.5% (6.5–16.2%)], root/root-nodules [by 31.0% (30.8–31.1%)] but did not affect N fixation in the lichens and leaves. Combinations of multiple environmental change factors stimulated N fixation in the soil/soil crusts [by 63.2% (57.5–69.2%)], leaf litter [by 901.6% (698.8–1155.7%)], mosses [by 93.0% (85.2–101.1%)] and inhibited N fixation in root/root-nodules [by 14.9% (14.7–15.1%)] but did not affect N fixation in the lichens. Overall, non-symbiotic and symbiotic N fixation had similar responses to individual environmental change factors, but they showed different responses to the combinations of multiple environmental change factors (Fig. S6).

Anthropogenic effects on N fixation under environmental change scenarios

The response ratios of N fixation to environmental change factors declined from unmanaged ecosystems to the ecosystems under disturbance (Fig. 3). Following warming treatment, the positive responses of N fixation were larger in unmanaged ecosystems and ecosystems in restoration than in the ecosystems under disturbance ($F_{2,143}$=10.0, p<0.001). Following treatments of elevated CO_2 and increased precipitation, the positive responses of N fixation were larger in unmanaged ecosystems than in the ecosystems in restoration and under disturbance [$F_{2,43}$=3.2, p=0.049 (for elevated CO_2); $F_{2,108}$=22.1, p<0.001 (for increased precipitation)]. Following treatment of increased drought, the responses of N fixation were higher in unmanaged ecosystems (positive responses) than in the ecosystems in restoration and under disturbance (negative responses; $F_{2,56}$=5.1, p=0.009). Following treatment of increased N deposition, the negative responses of N fixation were the smallest in unmanaged ecosystems, followed by the ecosystems under disturbance, and the largest in the ecosystems in restoration ($F_{2,245}$=17.9, p<0.001). Following treatment of multiple environmental change factors combined, the responses of N fixation were the highest in the ecosystems in restoration (positive responses), followed by unmanaged ecosystems, and the lowest in the ecosystems under disturbance (negative responses; $F_{2,59}$=5.8, p=0.005).

Changes in ecosystem characteristics and their relationships with N fixation responses

Human disturbance affected soil physicochemical properties (Fig. 4a–d). Among three types of ecosystems (no management, restoration, and disturbance), soil bulk density and clay + loam contents were the highest in the ecosystems under disturbance ($F_{2,241}$=40.8, p<0.001) and ecosystems in restoration ($F_{2,251}$=55.6, p<0.001), respectively. Soil pH was higher in unmanaged ecosystems and ecosystems in restoration than in the ecosystems under disturbance ($F_{2,493}$=27.5, p<0.001). In contrast, soil moisture contents did not differ among three types of ecosystems ($F_{2,287}$=0.6, p=0.552). Soil bulk density had positive relationships with the N fixation responses to warming (r^2=0.04, p<0.001; logarithmic regression models, hereafter) and increased drought (r^2=0.59, p<0.001), negative relationships with those to increased N deposition (r^2=0.04, p<0.001) and multiple environmental change factors combined (r^2=0.56, p<0.001), and no significant relationship with those to elevated CO_2 (p=0.45) and increased precipitation (p=0.45; Fig. 4e). Soil clay + loam contents had positive relationships with the N fixation responses to increased drought (r^2=0.04, p=0.002) and multiple environmental change factors combined (r^2=0.34, p<0.001), negative relationships with those to warming (r^2=0.06, p<0.001), increased precipitation (r^2=0.14, p<0.001), increased N deposition (r^2=0.02, p<0.001), and no significant relationship with those to elevated CO_2 (p=0.27; Fig. 4f). Soil moisture contents had positive relationships with the N fixation responses to increased drought (r^2=0.03, p=0.03), N deposition (r^2=0.03, p<0.001), and multiple environmental change factors combined (r^2=0.48, p<0.001), negative relationships with those to warming (r^2=0.33, p<0.001) and increased precipitation (r^2=0.36, p<0.001), and no relationship with those to elevated CO_2 (p=0.91; Fig. 4g). Soil pH had positive relationships with the N fixation responses to warming (r^2=0.38, p<0.001) and increased precipitation (r^2=0.10, p<0.001), negative relationships with those to increased N deposition (r^2=0.04, p<0.001), and no significant relationship with those to elevated CO_2 (p=0.11), increased drought (p=0.32), and multiple environmental change factors combined (p=0.46; Fig. 4h).

Human disturbance affected plant and microbial characteristics (Fig. 5a–c). Both dominant plant species numbers and soil MBC were the highest in unmanaged ecosystems and the lowest in the ecosystems under disturbance [$F_{2,649}$=72.7, p<0.001 (for dominant plant species numbers); $F_{2,129}$=16.2, p<0.001 (for soil MBC)]. Plant coverage was the highest in the ecosystems in restoration and the lowest in the ecosystems under disturbance ($F_{2,283}$=8.2, p<0.001). Dominant plant species numbers had positive relationships with the N fixation responses to elevated CO_2 (r^2=0.09, p=0.001), increased drought (r^2=0.19, p<0.001), N deposition (r^2=0.03, p=0.001), and multiple environmental change factors combined (r^2=0.16, p<0.001), and no relationship with those to warming (p=0.06) and increased precipitation (p=0.06; Fig. 5d). Plant coverage had positive relationships with the N fixation responses to increased precipitation (r^2=0.17, p=0.001) and multiple environmental change factors combined (r^2=0.09, p=0.02), and no relationship with those to warming (p=0.07), elevated CO_2 (p=0.88), increased drought (p=0.08), and N deposition (p=0.56; Fig. 5e). Soil MBC had positive relationships with the N fixation responses to increased N deposition (r^2=0.33, p<0.001) and multiple environmental change factors combined (r^2=0.49, p=0.003), and no relationship with those to warming (p=0.61), elevated CO_2 (p=0.36), and increased precipitation (p=0.71; Fig. 5f).

Similarly, human disturbance affected availability of soil resources (Fig. 6a–f). Both soil C and P contents were higher in unmanaged ecosystems and ecosystems in restoration than in the ecosystems under disturbance [$F_{2,483}$=13.3, p<0.001 (for soil C); $F_{2,264}$=7.8, p<0.001 (for soil P)]. Both soil N and NO_3^- contents were higher in the ecosystems in restoration than in unmanaged ecosystems and ecosystems under disturbance [$F_{2,482}$=9.6,

p<0.001 (for soil N); $F_{2,265}$=11.4, p<0.001 (for soil NO_3^-)]. Soil NH_4^+ contents were higher in the ecosystems under disturbance than in unmanaged ecosystems and ecosystems in restoration ($F_{2,272}$=4.8, p=0.009). Soil inorganic P contents were the highest in unmanaged ecosystems and the lowest in the ecosystems under disturbance ($F_{2,351}$=12.1, p<0.001). Total soil C contents had positive relationships with the N fixation responses to warming (r^2=0.07, p<0.001), elevated CO_2 (r^2=0.11, p=0.008), increased precipitation (r^2=0.23, p<0.001), increased drought (r^2=0.46, p<0.001), N deposition (r^2=0.06, p<0.001), and multiple environmental change factors combined (r^2=0.37, p<0.001; Fig. 6g). Total soil N contents had positive relationships with the N fixation responses to increased drought (r^2=0.22, p<0.001) and multiple environmental change factors combined (r^2=0.16, p=0.03), and no significant relationship with those to warming (p=0.06), elevated CO_2 (p=0.13), increased precipitation (p=0.06), and N deposition (p=0.20; Fig. 6h). Total soil P contents had positive relationships with the N fixation responses to increased precipitation (r^2=0.16, p=0.02), drought (r^2=0.11, p=0.03), N deposition (r^2=0.19, p<0.001), and multiple environmental change factors combined (r^2=0.48, p<0.001), and no significant relationship with those to warming (p=0.14) and elevated CO_2 (p=0.97; Fig. 6i). Soil NO_3^- contents had positive relationships with the N fixation responses to multiple environmental change factors combined (r^2=0.38, p=0.01), and no significant relationship with those to warming (p=0.09) and elevated CO_2 (p=0.49), increased precipitation (p=0.89), drought (p=0.45), and N deposition (p=0.60; Fig. 6j). Soil NH_4^+ contents had no significant relationship with the N fixation responses to warming (p=0.32), elevated CO_2 (p=0.63), increased precipitation (p=0.86), drought (p=0.15), N deposition (p=0.98), and multiple environmental change factors combined (p=0.07; Fig. 6k). Soil inorganic P contents had positive relationships with the N fixation responses to elevated CO_2 (r^2=0.34, p=0.008), increased precipitation (r^2=0.40, p<0.001), drought (r^2=0.10, p=0.002), and multiple environmental change factors combined (r^2=0.34, p=0.004), and no significant relationship with those to warming (p=0.94) and increased N deposition (p=0.06; Fig. 6l).

DISCUSSION

Among the control plots of the studied ecosystems (no management, restoration, and disturbance), we found that ecosystems under disturbance had the lowest rates of N fixation in the soil, litter, mosses, and lichens (i.e., non-symbiotic N fixation) but not in the root-nodules (i.e., symbiotic N fixation; Fig. 1), which partially supports our hypothesis that human disturbance inhibits terrestrial N fixation (*H1*). Our findings contrast to many previous findings that human activities (e.g., logging, irrigation, cropping, and mowing) have minor (Stuiver et al., 2015) or positive effects (Goh & Bruce, 2005; Keuter, Veldkamp, & Corre, 2014; Wheatley et al., 1995) on N fixation but supports several previous observations that human disturbance (e.g., logging or harvesting) reduces non-symbiotic N fixation in forests (Palviainen et al., 2005; Zheng et al., 2017). The reasons for the declines in non-symbiotic N fixation after disturbance may be related to the decreases in biomass of N fixers (Palviainen et al., 2005) and the loss of nutrients that support N fixation (Zheng et al., 2017). In contrast to non-symbiotic N fixation, human disturbance did not inhibit symbiotic N fixation rates (Fig. 1). This finding is not surprising because agricultural activities and greenhouse researches often utilize or cultivate legume plants of high N fixation capacities for high production (Herridge, Peoples, & Boddey, 2008; Fig. S2c).

Consistent with our hypothesis (*H2*), we found positive effects of warming (+152.7%), increased precipitation (+73.1%), and elevated CO_2 (+19.6%), and negative effects of increased drought (–30.4%) and N deposition (–31.0%) on N fixation at terrestrial biome scales (Fig. 2a, S5a). These findings agree with previous theories and views that (I) warm and wet conditions favor N fixers because N fixation is an enzymatic process (Houlton et al., 2008; Reed et al., 2011); (II) high C resource availability favors N fixers because N fixation is energetically expensive (Alberty, 2005; Vitousek & Hobbie, 2000); and (III) N fixers reduce energy cost on fixation when soil N is sufficient (Gutschick, 1981). Although the directions and/or magnitudes of N fixation in response to environmental change factors varied with the types of biomes, compartments, and N-fixing pathways (Fig. 2b−c, S5b−c, S6), we found that several natural ecosystems are sensitive to specific environmental change factors. For example, N fixers in boreal forests and deserts (characterized by low rainfall) had the strongest responses to increased precipitation and drought; N fixers in deserts (characterized by low organic matter contents) had the strongest responses to elevated CO_2; N fixers in boreal forests (characterized by low atmospheric N pollution) had the strongest responses to increased N deposition; and N fixers in boreal forests, grasslands, and polar deserts (characterized by low air temperature) had the strongest responses to warming (Fig. 2b). Since biological N fixation represents the dominant source of new N in many natural ecosystems, such as boreal forests, deserts, and grasslands (Cleveland et al., 1999), our findings indicate that global environmental change may have strong effects on N cycling and NPP in these 'sensitive' biomes, which deserves consideration in the modeling of terrestrial N cycling and the relationship between N fixation and ecosystem NPP.

As hypothesized (*H2*), combinations of multiple (i.e., 2–3) environmental change factors stimulated (63.2–144.0%) or inhibited (10.7–67.6%) terrestrial N fixation depending on the types of combined factors (Fig. 2a, S5a). However, when the positive-effect factors (warming, elevated CO_2, or increased precipitation) and negative-effect factors (increased N deposition or drought) were combined, we found declines in terrestrial N fixation overall (Fig. 2a, S5a). These findings provide new insights for our understanding and accurate estimate of terrestrial N fixation in a changing world (Davies−Barnard & Friedlingstein, 2020; Galloway et al., 2004; Meyerholt et al., 2016; Sullivan et al., 2014; Vitousek et al., 2013) given that multiple environmental change factors may occur in terrestrial ecosystems.

Furthermore, we found that the response ratios of N fixation to environmental change factors declined from unmanaged ecosystems to the ecosystems under disturbance (Fig. 3), which supports our hypothesis that human disturbance leads to declines in terrestrial N fixation under environmental change scenarios (*H3*). Although the levels of several environmental change treatments (e.g., warming rates, precipitation amounts, and N-addition rates) were divergent among different ecosystem types (Fig. S7), we ruled out the possibility that the difference in treatment levels accounted for the declines in N fixation responses (because treatment levels had no significant relationship with N fixation responses to environmental change factors; $p>0.05$; Fig. S8). Our findings provide the important lines of evidence that human disturbance suppresses the positive responses of N fixation to warming, elevated CO_2, and increased precipitation and it intensifies the negative responses of N fixation to increased drought and N deposition (Fig. 3). Given that biological N fixation is a key process by which ecosystems respond and adapt to environmental change (e.g., in forest ecosystems, N-fixing plants and/or microbes often down-regulate fixation rates after exogenous N input; Cusack et al., 2009; Zheng et al., 2016a), our findings indicate that human disturbance may to some extent prevent the adaptive capacity of ecosystems under global change scenarios.

To explore the mechanisms of human disturbance inhibiting N fixation under environmental change scenarios, we firstly tested the differences in soil physiochemical properties among three ecosystem types (no management, restoration, and disturbance). Compared with unmanaged ecosystems, we found that ecosystems under disturbance had higher soil bulk density and ecosystems in restoration had higher clay + loam contents (Fig. 4a–d). This result, however, could not explain the declines in N fixation responses because high soil bulk density and clay + loam contents often provide a large habitable space (e.g., a higher standing stock of soils) and a low oxygen environment for N fixers, both of which theoretically favor N fixation (Cusack et al., 2009; Reed et al., 2011; Robson & Postgate, 1980). This view is also supported by our results that increases in soil bulk density and clay + loam contents enhanced the N fixation responses to warming, increased drought, and/or multiple environmental change factors combined (Fig. 4e–f). Nevertheless, we found that increases in soil bulk density and clay + loam contents could explain 2–56% of the declines in N fixation responses to warming, increased precipitation, and/or N deposition (Fig. 4e–f). The reason for this result, however, is not clear due to limiting evidence available, and we suggest future studies to explore this phenomenon. In addition, we found that soil pH decreased from ~6.3 (in unmanaged ecosystems) to ~5.0 (in the ecosystems in restoration), which could explain 10–38% of the declines in N fixation responses to warming and increased drought (Fig. 4h). This mechanism could be supported by previous findings that N-fixing bacteria are adapted to neutral or slightly alkaline conditions (Mulder & Brotonegoro, 1974; Pham & Burgess, 1993) and their abundance decreases under acidic conditions (Limmer & Drake, 1996).

Our results showed that dominant plant species numbers and soil microbial biomass declined from unmanaged ecosystems to the ecosystems under disturbance (Fig. 5a, c), which explained 3−49% of the variations in N fixation responses (Fig. 5d, f). There are two reasons that account for this result. First, given that N fixation is the dominant N source for living organisms (e.g., non-N-fixing plants; Rousk et al., 2017c), the decreases in plant species and soil microbial biomass may reduce the ecosystem's demand for total fixed N. Second, plants can provide habitable environments for N fixers, such as canopy foliage (Moyes et al., 2016; Reed, Cleveland, & Townsend, 2008), tree trunks (Zheng et al., 2017), leaf litter (Reed et al., 2007a), rhizospheric soils (Zheng et al., 2016a), and root nodules (Menge & Hedin, 2009), and they also provide nutrients (e.g., via leaf leachate and litter decomposition) available to N fixers. Therefore, decreases in plant species numbers may damage the habitable environments of N fixers and inhibit N fixation, as supported by previous findings that forest logging reduced moss biomass and N fixation rates (Jurgensen, Graham, Larsen, & Harvey, 1992; Palviainen et al., 2005).

The declines in N fixation responses with human activities may be also attributed to the changes in soil resources. Among the tested resources, we found that decreases in soil C contents explained 6–46% of the declines in N fixation responses to all types of environmental change factors (Fig. 6a, g). This finding agrees with previous findings from forest ecosystems that labile C was an important driver of N fixation (Pérez, Carmona, & Armesto, 2010; Vitousek & Hobbie, 2000; Zheng, Chen, Li, Luo, & Mo, 2020) as well as recent hypotheses and observations that high organic C contents in canopy and litter layers stimulated N fixation despite soil N richness (Hedin, Brookshire, Menge, & Barron, 2009) and atmospheric N deposition (Zheng et al., 2018). Our finding reveals the importance of soil C in driving N fixation under environmental change scenarios and a potential C and N coupling relationship since the decreases in soil C inhibit N fixation and may eventually limit ecosystem NPP and C sequestration. Total and inorganic P in the soils decreased with human disturbance (Fig. 6c, f), which explained 10–48% of the declines in N fixation responses to all types of environmental change factors (except for warming; Fig. 6i, l). This result contrasts with previous observations that soil P alone did not limit N fixation (Barron et al., 2009; Perakis, Pett-Ridge, & Catricala, 2017; Vitousek, 1999) and is consistent with previous findings that P enrichment stimulated N fixation in forests (Reed et al., 2007a; Zheng et al., 2016b), grasslands (Reed et al., 2007b), and tundra (Rousk, Degboe, Michelsen, Bradley, & Bellenger, 2017a). Since soil P is in shortage in natural ecosystems (Hedin, Vitousek, & Matson, 2003) and human activities are accelerating terrestrial P limitation (Vitousek, Porder, Houlton, & Chadwick, 2010), our finding indicates that loss of soil P may constrain terrestrial N fixation. Total N and NO_3^- in the soils explained 16–38% of the variation in N fixation responses only under the scenarios of increased drought and/or multiple environmental change factors combined, and soil NH_4^+ could not explain the variation in N fixation responses (Fig. 6h, j). These results extend the 'leaky nitrostat' model that biological N fixation in certain ecosystem compartments (e.g., canopy foliage and forest floor) is less controlled by soil N richness (Hedin et al., 2009; Menge & Hedin, 2009) and indicate that soil N has a weak control over N fixation in response to environmental change.

Overall, there are several limitations of our study. First, although our dataset covers a wide range of terrestrial ecosystems (Fig. S1), relevant researches are very limited in certain biomes, such as tundra, heath, and shrublands (Fig. S9), which impedes our accurate understanding and modeling of N fixation in response to global change in these biomes. Second, because no published study has explored the combined effects of more than three environmental change factors on N fixation, our meta-analysis focuses on the combined effects of 2–3 environmental change factors. Given that N fixation and other ecological processes in terrestrial ecosystems are commonly affected by multiple global change factors, we suggest that future studies and experimental designs should incorporate more global change factors simultaneously. Third, although the variations in soil physicochemical properties, plant and microbial characteristics, and soil resources can partially explain the variations in N fixation in response to human disturbance and environmental change factors, many of the correlations are weak (Fig. 4−6). Due to data limitation, other important factors, such as micronutrients (e.g., molybdenum; Barron et al., 2009), light intensity (e.g., Taylor & Menge, 2018), and tree species (e.g., Reed et al., 2008) that may affect N fixation were not evaluated. Moreover, it is noted that several human-impacted systems (e.g., croplands) that are initially selected in certain areas with fertile soils or specific soil textures may confuse our understanding of anthropogenic effects on these soil properties. Thus, we suggest that empirical studies are needed to enhance our understanding of the mechanisms regarding human disturbance and environmental change regulating N fixation.

In summary, this study reveals for the first time how human disturbance activities and environmental change factors affect biological N fixation in terrestrial biomes. We found that (1) human disturbance inhibited non-symbiotic N fixation but not symbiotic N fixation; (2) warming, elevated CO_2, and increased precipitation stimulated N fixation, but increased drought, N deposition, and combinations of available multiple environmental change factors inhibited N fixation, and the extents of these effects varied among biomes and ecosystem compartments; and (3) human disturbance decreased the N fixation responses to environmental change factors, which could be partially explained by the changes in soil physicochemical properties and the declines in dominant plant species numbers, soil microbial biomass, and soil resources. Because our current understanding regarding the rates of and the controls on biological N fixation, a key pathway of new N inputs into Earth' ecosystems, remains very poor (Reed et al., 2011; Vitousek et al., 2013), our study revealing the effects of human disturbance activities and multiple environmental change factors on terrestrial N fixation as well as the mechanisms underlying these effects can improve our understanding, estimation, modeling, and prediction of terrestrial N budgets, NPP, and ecosystem feedbacks in a changing world.

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AUTHOR CONTRIBUTION

M.H.Z. and J.M.M. designed the study; M.H.Z. collected and analyzed the data; M.H.Z., Z.H.Z., P.Z., Y.Q.L., Q.Y., K.R.Z., L.S., and J.M.M. wrote the paper.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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FIGURE LEGENDS

Fig. 1 Nitrogen (N) fixation rates per unit mass **(a−c)** or per unit area **(d−f)** in three types of field ecosystems [ecosystems without management (No management), in restoration (Restoration), and under disturbance (Disturbance)]. 'Ndfa' represents the N that is derived from atmosphere. Solid hexagons and error bars represent the means and standard errors, respectively. Statistical significance among different ecosystem types is detected when *p* value is lower than 0.05 (as determined by one-way ANOVA). NA: not available.

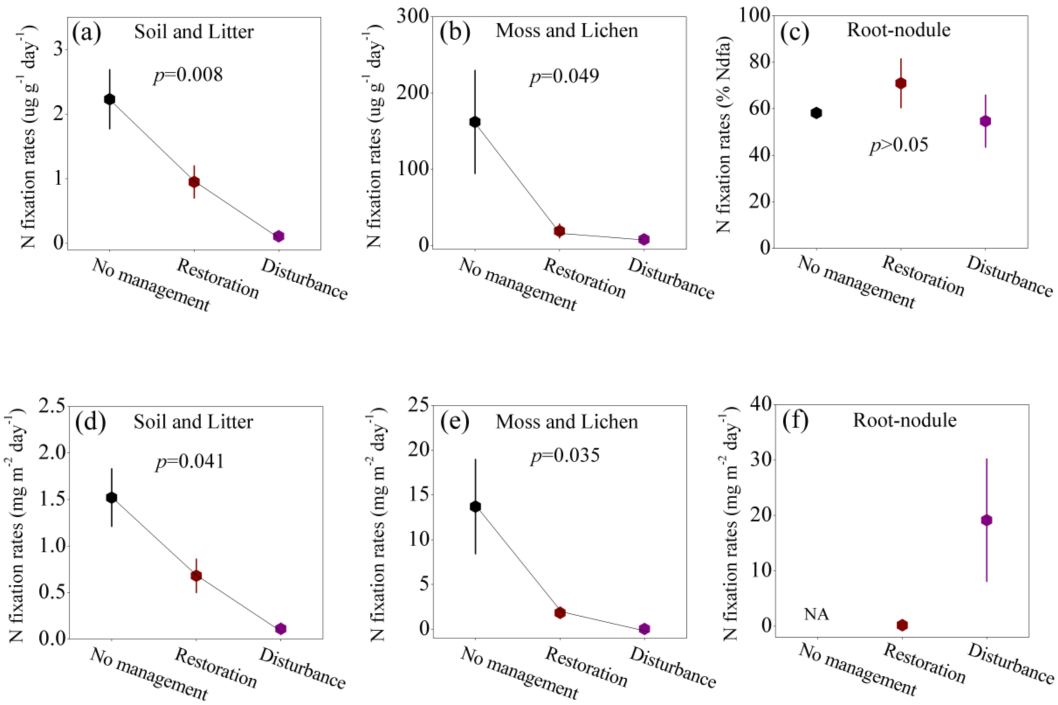
Fig. 2 Natural logarithm transformed response ratio (RR) of biological nitrogen fixation to environmental change factors overall **(a)** and in different biomes **(b)** and ecosystem compartments **(c)**. W, C, P, D, and N represent warming (blue color), elevated carbon dioxide (green color), increased precipitation (dark-yellow color), increased drought (orange color) and increased nitrogen deposition (red color), respectively. 'Multiple environmental change factors' represents the combinations of at least two environmental change factors, including W+P, W+D, W+C+D, C+D, C+N, C+W, and N+P (black color). 'Overall' represents the overall effects of available multiple environmental change factors on biological nitrogen fixation. Each solid circle and error bar represent weighted mean RR and 95% confidence interval (CI), respectively. The numbers in brackets represent sample sizes. Horizontal dashed line is the reference of the response ratio of zero. Significant responses ($p<0.05$) are recognized if the 95% CI does not overlap with zero.

Fig. 3 Natural logarithm transformed response ratio (RR) **(a)** and untransformed RR **(b)** of biological nitrogen fixation to environmental change factors in three types of field ecosystems [ecosystems without management (No management), in restoration (Restoration), and under disturbance (Disturbance)]. The numbers in brackets represent sample sizes. Horizontal dashed line is the reference of the response ratio of zero. Different lowercase letters represent significant difference ($p<0.05$) among different types of ecosystems (as determined by one-way ANOVA). 'Multiple environmental change factors' represents the combinations of at least two environmental change factors.

Fig. 4 Soil physicochemical properties (bulk density **(a)**, clay + loam contents **(b)**, moisture **(c)**, and pH **(d)**) in three types of field ecosystems [ecosystems without management (No management), in restoration (Restoration), and under disturbance (Disturbance)] and their relationships with the natural logarithm transformed response ratio of biological nitrogen fixation to environmental change factors **(e–h)**. Different lowercase letters represent significant difference among different ecosystems (p<0.05). W, C, P, D, N, and M represent warming (blue color), elevated carbon dioxide (green color), increased precipitation (dark-yellow color), increased drought (orange color), increased nitrogen deposition (red color), and multiple environmental change factors (black color), respectively.

Fig. 5 Dominant plant species number **(a)**, plant coverage **(b)**, and soil microbial biomass carbon (MBC) **(c)** in three types of field ecosystems [ecosystems without management (No management), in restoration (Restoration), and under disturbance (Disturbance)] and their relationships with the natural logarithm transformed response ratio of biological nitrogen fixation to environmental change factors **(d–f)**. Different lowercase letters represent significant difference among different ecosystems (p<0.05). W, C, P, D, N, and M represent warming (blue color), elevated carbon dioxide (green color), increased precipitation (dark-yellow color), increased drought (orange color), increased nitrogen deposition (red color), and multiple environmental change factors (black color), respectively. NA: not available, due to limiting data (n<3) for regression analysis.

Fig. 6 Soil resources (carbon **(a)**, nitrogen **(b)**, phosphorus **(c)**, nitrate **(d)**, ammonium **(e)**, and inorganic phosphorus **(f)**) in three types of field ecosystems [ecosystems without management (No management), in restoration (Restoration), and under disturbance (Disturbance)] and their relationships with the natural logarithm transformed response ratio of biological nitrogen fixation to environmental change factors **(g–l)**. Different lowercase letters represent significant difference among different ecosystems (p<0.05). Soil C, N, P, NO_3^-, NH_4^+ and IP represent soil carbon, nitrogen, phosphorus, nitrate, ammonium, and inorganic phosphorus, respectively. W, C, P, D, N, and M represent warming (blue color), elevated carbon dioxide (green color), increased precipitation (dark-yellow color), increased drought (orange color), increased nitrogen deposition (red color), and multiple environmental change factors (black color), respectively.



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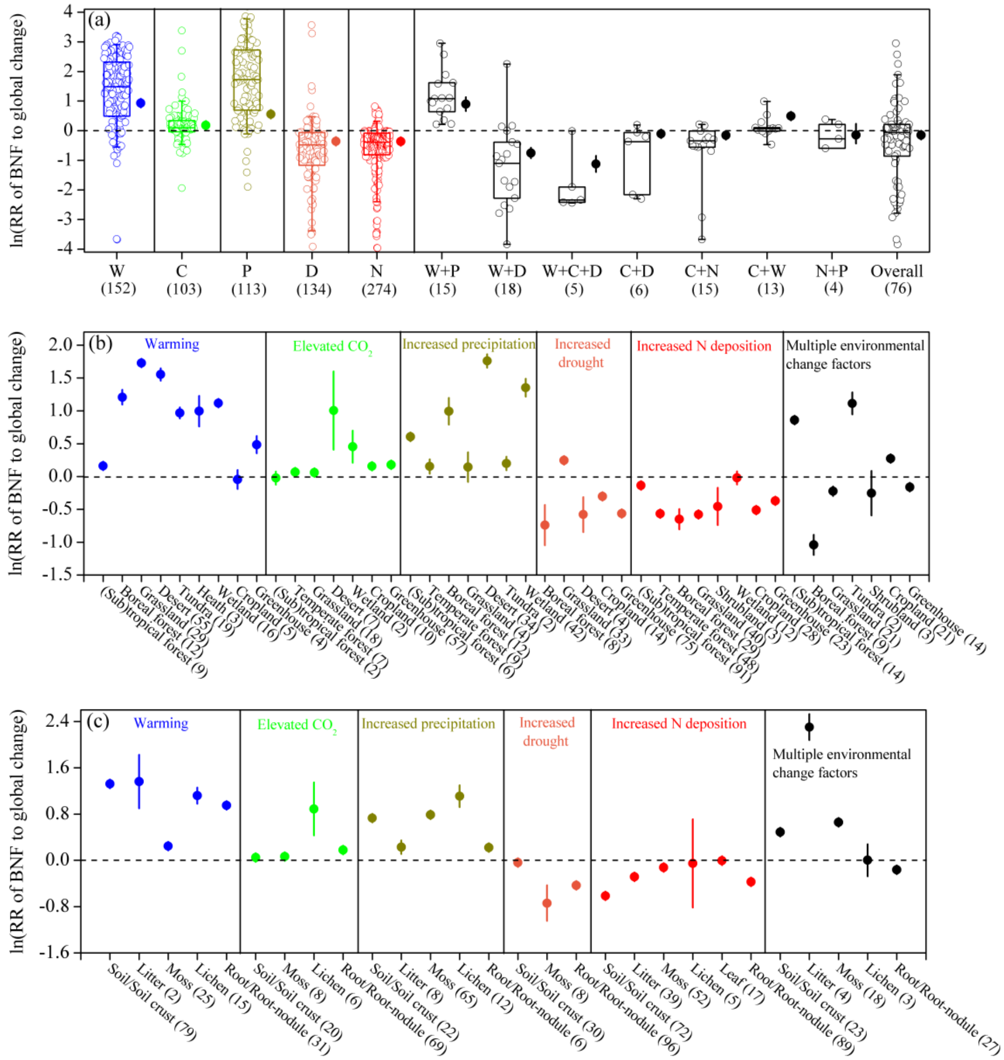

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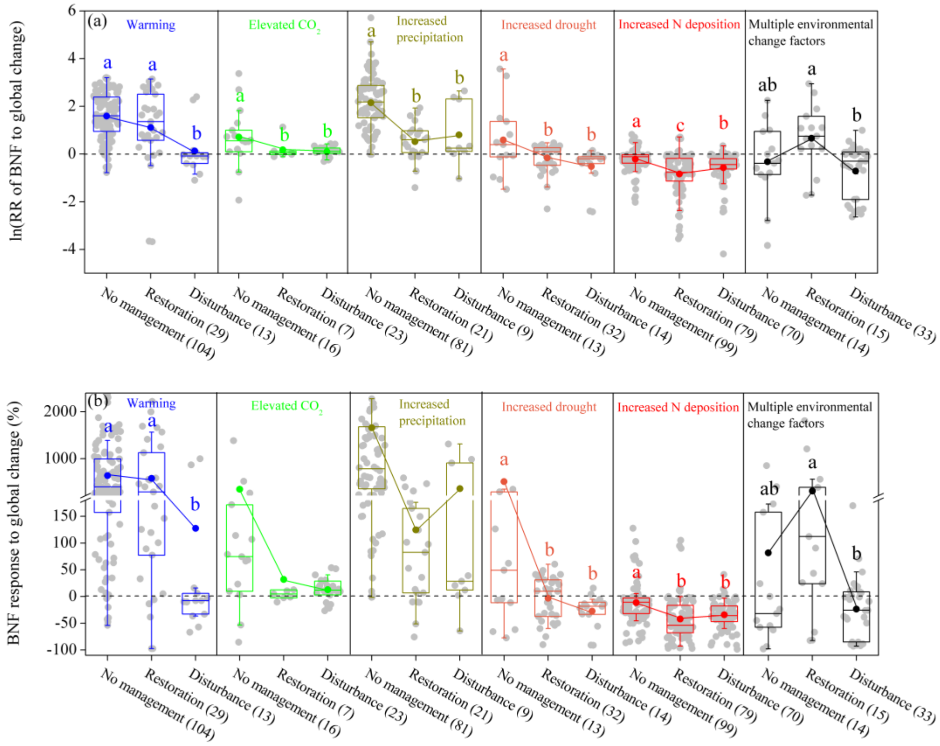

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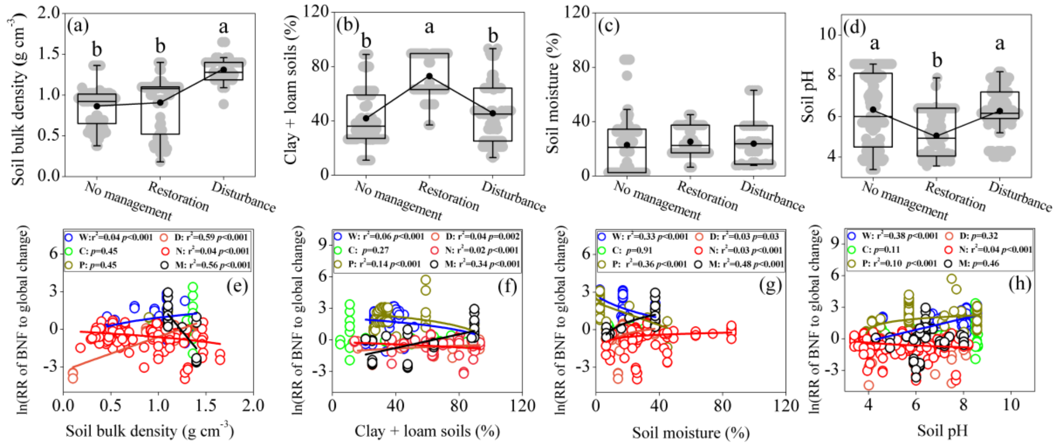

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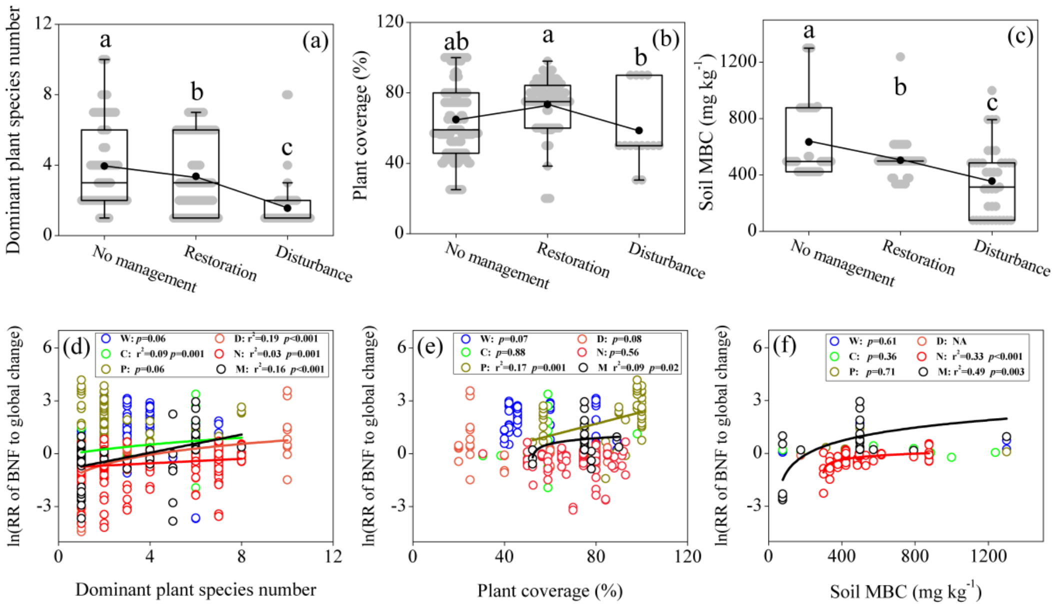

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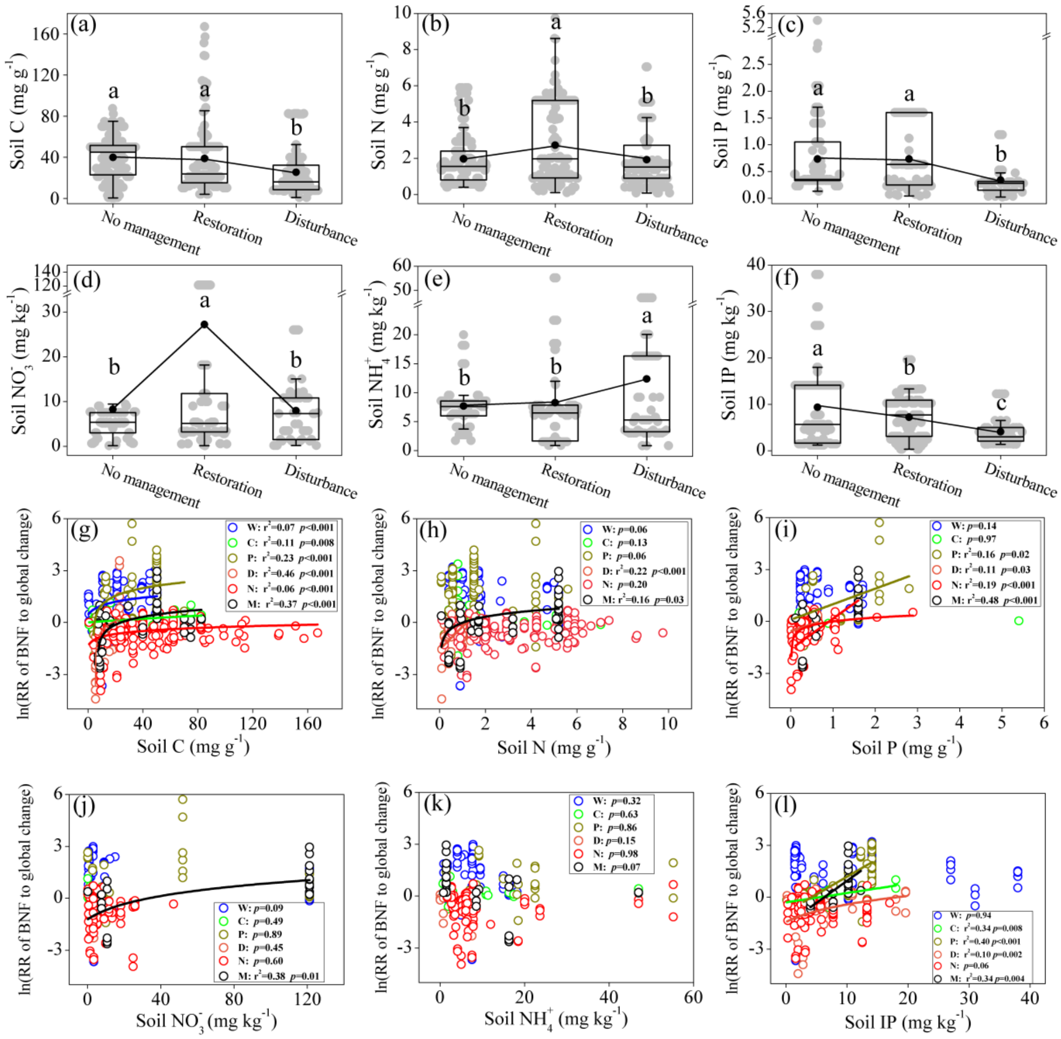

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