

The gap between atmospheric nitrogen deposition experiments and reality

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Abstract

Anthropogenic activities have dramatically altered the global nitrogen (N) cycle. Atmospheric N deposition, primarily from combustion of biomass and fossil fuels, has caused acidification of precipitation and freshwater and triggered intense research into ecosystem responses to this pollutant. Experimental simulations of N deposition have been the main scientific tool to understand ecosystem responses, revealing dramatic impacts on soil microbes, plants, and higher trophic levels. However, comparison of the experimental treatments applied in the vast majority of studies with observational and modelled N deposition reveals a wide gulf between research and reality. While the majority of experimental treatments exceed $100 \text{ kg N ha}^{-1} \text{ y}^{-1}$, global median land surface deposition rates are only around $1 \text{ kg N ha}^{-1} \text{ y}^{-1}$ and only exceed $10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ in certain regions, primarily in China. Experimental N deposition treatments are in fact similar to mineral fertilizer application rates in agriculture. Some ecological guilds, such as saprotrophic fungi, are highly sensitive to N and respond differently to low and high N availability. In addition, very high levels of N application cause changes in soil chemistry, such as acidification, meaning that unrealistic experimental treatments are unlikely to reveal true ecosystem responses to N. Hence, despite decades of research, past experiments can tell us little about how the biosphere has responded to anthropogenic N deposition. A new, and more realistic, approach is required if experimental studies are to contribute to our understanding of this important phenomenon.

27 Introduction

28 Anthropogenic activities have dramatically altered the global biogeochemical cycling of nitrogen (N).
29 The Earth's atmosphere is composed mainly of biologically-inert N₂ gas, which must be oxidized or
30 reduced to become reactive N and available to the biosphere. Biological N fixation by symbiotic and
31 free-living bacteria delivers 58 ± 29 Tg N yr⁻¹ to terrestrial ecosystems, 140 ± 70 Tg N yr⁻¹ to marine
32 ecosystems, and 60 ± 18 Tg N yr⁻¹ to agricultural systems, all as reduced N, NH_x (Fowler *et al.*,
33 2013). Industrial production of ammonia via the Haber Bosch process generates 120 ± 12 Tg N yr⁻¹,
34 compared with a mean estimate of 258 Tg N yr⁻¹ for combined microbial fixation. Oxidized N, NO_y,
35 is generated by lightning (5 ± 2.5 Tg N yr⁻¹) and combustion of fossil fuels (30 ± 3 Tg N yr⁻¹). Hence,
36 total annual fixation of N is around 413 Tg N yr⁻¹ but with very large uncertainties, of which around
37 half is due to human activities (Fowler *et al.*, 2013). Fixed N passes through a complex series of
38 chemical and biochemical transformations before returning to the atmosphere as molecular N.
39 Reactive N is either sequestered by plants and microbes for protein synthesis, or metabolized by
40 nitrification or denitrification to various gaseous forms. Hence, either through fixation of N by
41 lightning or fossil fuel combustion, or microbial conversion of N in organic matter to NH_x or NO_y, the
42 atmosphere contains a significant concentration of reactive N. Dry or wet deposition of this reactive N
43 carries around 70 Tg N yr⁻¹ to the land surface and 30 Tg N yr⁻¹ to the oceans, though with
44 considerable uncertainty (Fowler *et al.*, 2013). Wet deposition occurs by solution in precipitation,
45 while dry deposition occurs through direction adsorption onto plant and soil surfaces, diffusion into
46 plant stomata, and adsorption into particles that are then deposited (Hanson & Lindberg, 1991).

47 Reactive N is vital to life and many reactive N compounds have chemical properties (e.g. forming
48 acidic solutions) that can affect biological processes. The potential for diverse impacts of N
49 deposition on ecosystems have been recognized for many decades (Likens *et al.*, 1972; Almer *et al.*,
50 1974; Söderlund, 1977). Describing and quantifying the responses of ecosystems to N deposition, or
51 'nitrogen pollution', has been among most intensively studied areas of global change research. This
52 corpus has revealed pervasive effects of N deposition on soil microbes (Zhang *et al.*, 2018; Cheng *et al.*
53 *et al.*, 2019), plants (Du & de Vries, 2018; Schulte-Uebbing & de Vries, 2018), and higher trophic levels
54 in terrestrial ecosystems (Stevens *et al.*, 2018). In contrast, biogeochemical cycle models which
55 include negative feedbacks of N fixation suggest that marine ecosystems show limited responses of
56 productivity to N deposition (Somes *et al.*, 2016).

57 In terrestrial ecosystems, the concept of critical loads has been used to monitor the potential impacts
58 of N deposition. The critical load is "The highest load that will not cause chemical changes leading to
59 long-term harmful effects on most sensitive ecological systems" (Nilsson, 1988). Critical loads are
60 related to N saturation levels, whereby N-limited ecosystem processes such as plant growth absorb
61 additional N deposition. Experimental evidence suggests that N saturation for aboveground net
62 primary production is 50–60 kg N ha⁻¹ yr⁻¹ (Tian *et al.*, 2016). Critical loads have proven useful policy

63 tools, allowing agencies to monitor the occurrence of potentially harmful levels of N deposition while
64 taking the varying sensitivities of different ecosystems into account (Pardo *et al.*, 2011). Hence,
65 policies to manage pollution from N deposition require understanding of the rate of N deposition, the
66 critical load of the ecosystem, and the effects of varying N availability on different organisms and
67 ecosystem functions. Controlled experiments that manipulate N levels and evaluate ecosystem
68 responses are key to understanding the effects of N deposition and making informed policy decisions.
69 However, to be of value, these experiments must employ experimental treatments that mimic realistic
70 current or potential future deposition rates. Here, I will demonstrate that our understanding of the
71 effects of N deposition on natural ecosystems has been skewed by unrealistic experimental treatments
72 that often greatly exceed deposition levels found in even the most heavily polluted settings.

73 **Global N deposition rates**

74 Ground-based and remote sensing measurements, coupled with biogeochemical and atmospheric
75 transport models, provide estimates of global N deposition rates. In the following discussion, all
76 deposition rates will be reported as $\text{kg N ha}^{-1} \text{y}^{-1}$. While not strictly in SI units, this measure is most
77 commonly used in the literature. The Inter-Sectoral Impact Model Intercomparison Project (ISIMIP)
78 provides researchers with a consistent portfolio of datasets for assessing global change (Warszawski
79 *et al.*, 2014). N deposition data used in ISIMIP2b simulations are derived from the Atmospheric
80 Chemistry and Climate Model Intercomparison Project (ACCMIP) (Lamarque *et al.*, 2013a,b),
81 providing monthly and annual estimates of total (wet + dry) NH_x and NO_y deposition at 0.5° spatial
82 resolution. This model suggests median land surface deposition of $0.54 \text{ kg N ha}^{-1} \text{y}^{-1}$ in 1861, with 99
83 per cent of the land surface receiving less than $3.7 \text{ kg N ha}^{-1} \text{y}^{-1}$ (Fig. 1, Fig. S1). In 2021, the model
84 predicts median land surface deposition of $1.2 \text{ kg N ha}^{-1} \text{y}^{-1}$, with 99 per cent of the land surface
85 receiving less than $14.2 \text{ kg N ha}^{-1} \text{y}^{-1}$ (Fig. 2). The highest deposition rates are in Kalimantan,
86 southern Borneo. By 2081, under the RCP6.0 emissions scenario, median deposition is projected to
87 increase slightly to $1.3 \text{ kg N ha}^{-1} \text{y}^{-1}$, with 99 per cent of the land surface receiving less than 16.0 kg N
88 $\text{ha}^{-1} \text{y}^{-1}$. The models suggest that less than 3 per cent of the land currently receives more than 10 kg N
89 $\text{ha}^{-1} \text{y}^{-1}$, with a similar distribution in 2081.

90 The GEOS-Chem Chemical Transport Model, using the MERRA-2 meteorological reanalysis dataset
91 (Gelaro *et al.*, 2017) and Emission Database for Global Atmospheric Research (EDGAR v4.2
92 edgar.jrc.ec.europa.eu/overview.php?v=42), estimated a global (land and sea) average of 1.84 kg N
93 $\text{ha}^{-1} \text{y}^{-1}$ in 2016 at a spatial resolution of 2.5° longitude \times 2° latitude (Ackerman *et al.*, 2019).
94 Considering only land surface pixels, the model estimates median deposition of $1.09 \text{ kg N ha}^{-1} \text{y}^{-1}$
95 (IQR 0.01–3.37) in 1984 and $1.46 \text{ kg N ha}^{-1} \text{y}^{-1}$ (IQR 0.01–3.65) in 2016. For 2016, median dry
96 deposition was estimated at $0.49 \text{ kg N ha}^{-1} \text{y}^{-1}$ (IQR 0.001–1.48), while median wet deposition was
97 $0.78 \text{ kg N ha}^{-1} \text{y}^{-1}$ (IQR 0.007–2.21). The highest level was $51.55 \text{ kg N ha}^{-1} \text{y}^{-1}$ in Central China, with

98 hotspots in eastern Asia, Europe, eastern North America and southern Brazil (Ackerman *et al.*, 2019).
99 Deposition increased dramatically (by around $1 \text{ kg N ha}^{-1} \text{ y}^{-1}$) in eastern China between 1984 and
100 2016, with subtler changes elsewhere. Central China was the only region to exceed $30 \text{ kg N ha}^{-1} \text{ y}^{-1}$. A
101 detailed analysis of GEO-Chem estimates for China found mean deposition of $16.4 \text{ kg N ha}^{-1} \text{ y}^{-1}$
102 across the country, and in total is around half the N input of fertilizer application (Zhao *et al.*, 2017).
103 Historical and potential future N deposition have also been investigated using the TracerModel 4 of
104 the Environmental Chemical Processes Laboratory (TM4-ECPL) (Kanakidou *et al.*, 2016). TM4-
105 ECPL accounts for inorganic and organic N sources in gaseous and particulate phases, and was driven
106 by Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP) historical and
107 future RCP6.0 and RCP8.5 emissions scenarios. Total global terrestrial N deposition was estimated as
108 48 Tg N y^{-1} in 1850, 126 Tg N y^{-1} in 2005, and 132 Tg N y^{-1} in 2050 under the RCP6.0 scenario.
109 Deposition rates per unit area are not given explicitly, but maps indicate between $10\text{--}50 \text{ kg N ha}^{-1} \text{ y}^{-1}$
110 in eastern USA, much of Europe, India and China, with $1\text{--}10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ across other land areas in
111 2005 (Kanakidou *et al.*, 2016). Interpolation of ground measurements, combined with satellite remote
112 sensing of NO_2 , estimated total global deposition of 34.3 Tg N y^{-1} from 2005-2014 (Jia *et al.*, 2016),
113 considerably lower than the TM4-ECPL model (Kanakidou *et al.*, 2016). Global average deposition
114 rates for NH_3 , NO_2 , HNO_3 , NH_4^+ and NO_3^- were 1.64, 0.45, 0.27, 0.11, and $0.02 \text{ kg N ha}^{-1} \text{ y}^{-1}$,
115 respectively. Ground measurement sites were concentrated in regions with the highest deposition
116 rates, i.e. eastern USA, Europe and eastern China.

117 China has among the highest N deposition rates globally, due to rapid growth of fossil fuel
118 combustion and agricultural intensification over recent decades (Yu *et al.*, 2019). Spatial interpolation
119 of observational data suggest that the highest levels of total N deposition occur in south-eastern and
120 coastal regions, exceeding $50 \text{ kg N ha}^{-1} \text{ y}^{-1}$. Over the whole country, mean deposition between 2011
121 and 2015 was $20.4 \pm 2.6 \text{ kg N ha}^{-1} \text{ y}^{-1}$, with approximately equal contributions from dry and wet
122 deposition, and around two thirds contributed by NH_x and one third NO_y (Yu *et al.*, 2019). Total
123 deposition rates have stabilized, increasing from around $14 \text{ kg N ha}^{-1} \text{ y}^{-1}$ in 1985, with NO_y continuing
124 to increase and NH_x declining slightly in recent years.

125 Observational data from the European Air Chemistry Network (EACN) and European Monitoring and
126 Evaluation Programme (EMEP) combined with two chemical transport models suggest that average
127 total N deposition across Europe has declined from $10.3 \text{ kg N ha}^{-1} \text{ y}^{-1}$ in 1990 to $6.6 \text{ kg N ha}^{-1} \text{ y}^{-1}$ in
128 2018 (Engardt *et al.*, 2017; Schmitz *et al.*, 2019). Between 2000 and 2015, central European and
129 Scandinavian forests saw declines in N throughfall deposition of more than 30 per cent (Schmitz *et*
130 *al.*, 2019). What effect these declining deposition rates will have on forest ecosystems is not clear,
131 because very few experimental studies have investigated any hysteresis effects, and because rates and
132 levels of change in experimental studies are generally faster and greater than those experienced by
133 ecosystems (Schmitz *et al.*, 2019). Observations from 37 sites across the USA from 2011 to 2013

134 show total deposition rates ranging from 1.5 kg N ha⁻¹ y⁻¹ in Washington to 12.1 kg N ha⁻¹ y⁻¹ in the
135 Upper Midwest (Li *et al.*, 2016). As in Europe, N deposition has declined across the USA in recent
136 years. Total wet deposition decreased by an average of -0.036 kg N ha⁻¹ y⁻² between 1985 and 2012
137 (Du, 2016).

138 Experimental research on natural ecosystem responses to N deposition has commonly focussed on
139 forests (Knorr *et al.*, 2005; Zhang *et al.*, 2018; Cheng *et al.*, 2019; Schmitz *et al.*, 2019). Schwede *et al.*
140 *al.* (2018) compared two global N deposition estimates with high resolution land use maps to
141 investigate variation in deposition rates among ecosystems. The Task Force on Hemispheric
142 Transport of Air Pollution (HTAP2) combined results from eleven CTMs at 1.0° spatial resolution,
143 producing a multi-model mean deposition over forested pixels of 6.0 kg N ha⁻¹ y⁻¹ when using Global
144 Forest Monitoring Project data and 5.3 kg N ha⁻¹ y⁻¹ when using the GLC2000 Global Land Cover
145 map (Schwede *et al.*, 2018). The GLC2000 data indicated that the lowest mean deposition rates were
146 in semi-natural vegetation and grasslands (4.3 kg N ha⁻¹ y⁻¹), followed by forests and then croplands
147 (12.5 kg N ha⁻¹ y⁻¹). The European Monitoring and Evaluation Programme EMEP MSC-W model was
148 used to estimate deposition across different forest types, giving mean deposition of 7.1 kg N ha⁻¹ y⁻¹
149 across all forests, 1.2 kg N ha⁻¹ y⁻¹ in boreal forests, 7.2 kg N ha⁻¹ y⁻¹ in tropical forests, 7.3 kg N ha⁻¹
150 y⁻¹ in temperate forests and 14.6 kg N ha⁻¹ y⁻¹ in subtropical forests (Schwede *et al.*, 2018). The high
151 deposition rate in subtropical forests was driven largely by China. Further, in forest ecosystems the
152 tree canopy intercepts a large fraction, up to 80 per cent, of N deposition (Gaike *et al.*, 2007;
153 Sievering *et al.*, 2007; Wortman *et al.*, 2012). Thus, direct deposition of N onto forest soils will be
154 lower than expected by atmospheric models or open field measurements.

155 **Experimental N deposition rates**

156 Taken together, observational data and models suggest that land surface N deposition rates rarely
157 exceed 10 kg N ha⁻¹ y⁻¹, and that most of the area with greater deposition rates is in China. These
158 values can be compared to experimental deposition rates used to understand how anthropogenic N
159 affects natural and managed ecosystems. An experimental study on wood decay by basidiomycete
160 fungi, published nearly a decade ago (Bebber *et al.*, 2011), argued that most previous research had
161 employed unrealistically high deposition rates leading to conclusions of suppressed fungal activity
162 under elevated N load. The study took place at a woodland site in southern UK, experiencing a
163 deposition rate of 2.9 kg ha⁻¹ yr⁻¹ as NH₄⁺ and 0.7 kg ha⁻¹ yr⁻¹ as NO₃⁻. The experimental treatment was
164 equivalent to an additional 2.8 kg N ha⁻¹ yr⁻¹ as NO₃⁻, a 78 % increase. After 10 months, wood decay
165 and mycelial development were greater for wood blocks treated with additional N. Compared with
166 fungal mycelium, wood is N-poor (approximately 0.1 % dry mass of fresh wood compared with
167 approximately 1.5 % dry mass of mycelium), and fungi forage to acquire N and other nutrients
168 (Bebber *et al.*, 2011). Visualizations of radio-labelled amino acid analogues have demonstrated the

169 efficient scavenging of N and transport to carbon sinks (i.e., wood) by mycelial networks (Tlalka *et al.*, 2007; Fricker *et al.*, 2008). The increased decay rates seen under additional N deposition were
170 interpreted as enhanced metabolic rates possible when growth-limiting N become more available
171 (Bebber *et al.*, 2011). This experimental result supported earlier observational studies demonstrating
172 enhanced decomposition rates along N deposition gradients (Fenn & Dunn, 1989; Kuperman, 1999),
173 and with wood N content (Weedon *et al.*, 2009). Very early experimental studies showed that wood
174 decomposition rates increase with low rates of N addition (particularly as amino acid), but decline
175 with high rates of addition (Findlay, 1934). As argued (Bebber *et al.*, 2011), more recent experimental
176 studies have employed very high levels of simulated N deposition, which exceeded background
177 deposition rates by between 2.3 and 1000 times (Knorr *et al.*, 2005). However, the application of high
178 experimental N deposition treatments to investigate effects on wood decay fungi has continued (e.g.
179 Zak *et al.*, 2019).

181 A recent meta-analysis of N deposition effects on soil microbes further illustrates the issue of
182 unrealistic experimental treatments (Zhang *et al.*, 2018). The analysis included 1408 paired
183 (treatment-control) observations from 151 studies, concluding that N addition reduces total microbial
184 biomass, bacterial biomass, biomass carbon and microbial respiration. However, there are very large
185 disparities between control (background) deposition and treatment N addition rates within these
186 studies (Fig. 3). The median background deposition rate was 15.0 kg N ha⁻¹ y⁻¹ (interquartile range
187 6.2–16.0 kg N ha⁻¹ y⁻¹), while median experimental addition rate was 100.0 kg N ha⁻¹ y⁻¹ (IQR 50.0–
188 169.75 kg N ha⁻¹ y⁻¹) (Fig. 3a). The median total experimental deposition rate (i.e. background plus
189 additional) was 9.0 (IQR 5.5–25.5) times greater than the background deposition rate, with the most
190 extreme treatment being 1000 times greater than background (Fig. 3b,c). For comparison, a meta-
191 analysis of the effects of fertilizer use on soil microbes found a median mineral (NPK) fertilizer
192 addition rate of 80 kg N ha⁻¹ y⁻¹ (IQR 62.14–150.0 kg N ha⁻¹ y⁻¹) (Bebber & Richards, 2020).
193 Agricultural N fertilization rates in the UK are around 110 kg N ha⁻¹ y⁻¹ from mineral fertilizers and 9
194 kg N ha⁻¹ y⁻¹ from organic fertilizers (DEFRA, 2020). In summary, the experimental N deposition
195 rates in studies reported by Zhang *et al.* (2018) were similar to agricultural N fertilization and an order
196 of magnitude greater than background deposition rates.

197 Zhang *et al.* (2018) fitted linear regressions of N deposition rate to effect sizes, finding that bacterial
198 biomass, fungal biomass, microbial biomass carbon and total microbial biomass all decline with
199 increasing N. Here, I recalculate effect sizes and weights to ascertain whether these responses are
200 truly linear, and whether negative effects on microbial biomass occur under realistic N deposition
201 rates. As in Zhang *et al.* (2018), the effect size was calculated as the log response ratio $\ln(x_t / x_c)$ where
202 x_t and x_c are the mean values for treatment and control in a particular comparison. However, rather
203 than using a function of the number of replications alone, the weighting for each comparison was
204 equal to the inverse variance ($w = 1 / v$) (Lajeunesse, 2015):

$$v = \frac{s_t^2}{n_t^2 x_t^2} + \frac{s_c^2}{n_c^2 x_c^2}$$

206 where n is the number of replicates and s is the reported standard deviation of the mean.

207 Generalized additive models (GAMs) for four variables with the largest numbers of contributing
208 studies reveal a varying response to experimental N deposition/fertilization (Fig. 4). In most cases, the
209 AIC was smaller for the GAM than for the linear model, indicating sufficient evidence for a non-
210 linear response. For bacterial biomass, AIC is 109.2 for the linear model and 106.0 for the GAM. For
211 total biomass, AIC was 125.6 vs. 93.9, respectively. For microbial biomass carbon, AIC was 473.9 vs.
212 453.9, respectively. Only for fungal biomass did the GAM algorithm converge on a linear response.

213 Evidence for negative effects on bacterial biomass, fungal biomass and total biomass is only
214 significant once experimental deposition rates exceed around 30–100 kg N ha⁻¹ y⁻¹ (Fig. 4). There are
215 too few studies, and correspondingly high variances, to draw firm conclusions at more realistic rates
216 of N deposition. Linear regressions (vs. log-transformed N deposition rates) appear to be supported
217 for bacterial and fungal biomass. Fungal biomass is elevated for several of the less intensively
218 fertilized experiments, but there is insufficient evidence to determine whether more realistic
219 deposition rates would increase fungal biomass. Hence, the conclusion that N deposition reduces
220 microbial biomass only applies to very high levels of N addition.

221 As discussed by Zhang *et al.* (2018), other meta-analyses have found increased microbial biomass
222 carbon under experimental N deposition in agricultural systems (Geisseler & Scow, 2014), but
223 decreases in microbial biomass in unmanaged ecosystems (Treseder, 2008; Liu & Greaver, 2010; Lu
224 *et al.*, 2011a). Similarly, high experimental N deposition rates were found to suppress organic matter
225 decomposition in forest soils (Janssens *et al.*, 2010). Treatment and background rates (where reported)
226 in previous meta-analyses illustrate the gap between experiment and reality (Fig. 5). Here, data from
227 meta-analyses that do not explicitly focus on atmospheric N deposition effects, but rather on some
228 other aspect of N effects are not considered (e.g. LeBauer & Treseder, 2008; Vadeboncoeur, 2010; Lu
229 *et al.*, 2011b; Maynard *et al.*, 2014). Knorr *et al.* (2005) published background and treatment N
230 deposition rates for most of the studies they analysed, while Treseder (2008) estimated background
231 deposition rates using an interpolated observational dataset (Holland *et al.*, 2004). Janssens *et al.*
232 (2010) published only wet deposition background data, while many other meta-analyses did not
233 publish background deposition data. Lu *et al.* (2011a) published neither background deposition nor
234 treatment rates for the studies in their meta-analyses, but reported that experiments conducted in
235 natural ecosystems utilized somewhat lower addition rates (117 kg N ha⁻¹ y⁻¹) than those in
236 agricultural systems (149 kg N ha⁻¹ y⁻¹). Some meta-analyses of N deposition effects published no
237 background or treatment data (e.g. Nave *et al.*, 2009; Zhou *et al.*, 2014; Tian *et al.*, 2016). Most
238 recently, Zhou *et al.* (2020) published a comprehensive meta-analysis of the effects of global change
239 factors on soil microbial diversity, with data on N addition treatments in agricultural and natural

240 ecosystems. In each of these meta-analyses, N additions are around an order of magnitude greater
241 than background rates, and similar to agricultural fertilization rates. Background deposition rates in
242 these studies, where reported, tend to be near the upper limit of modelled global land surface
243 estimates (Fig. 5).

244 High levels of inorganic N fertilization affect many aspects of environmental chemistry, in addition to
245 enhancing N availability. Among the most widely known, due to the phenomenon of ‘acid rain’ and
246 associated mortality of forest trees and freshwater biota, is a decrease in pH (Likens *et al.*, 1972;
247 Almer *et al.*, 1974; Söderlund, 1977). Ammonia and ammonium are most likely to decrease soil pH
248 through release of protons on conversion to nitrate via the process of nitrification. The effects of
249 global change factors, like N deposition, on soil microbial diversity, are largely explained by the
250 effects of those factors on soil pH (Zhou *et al.*, 2020). N fertilization strongly decreases soil pH, and
251 hence indirectly affects soil ecosystem functioning (Zhou *et al.*, 2020). Positive effects of increased N
252 availability on soil microbial communities via enhanced plant productivity and belowground carbon
253 allocation are offset by acidification, in experiments with high levels of N addition (Chen *et al.*,
254 2015). Such unrealistic application rates may tell us more about pH effects than of N availability.

255 **Comparison with other global change experimental systems**

256 Perspective on the relative rates of experimental vs. actual N deposition can be gained by comparison
257 with other experimental systems in global change research. In the field of climate change impacts, soil
258 warming and free-air carbon dioxide enrichment (FACE) experiments are among the most common.
259 Globally-averaged combined land and ocean surface temperatures increased by 0.85 °C over the
260 period 1880 to 2012, driven largely by atmospheric CO₂ concentration rise from around 280 to 400
261 ppm (IPCC, 2014). The rate of future rises in atmospheric greenhouse gas concentrations will depend
262 upon global socioeconomic changes, which have been modelled in so-called Shared Socio-Economic
263 Pathway (SSP) scenarios (Meinshausen *et al.*, 2020). Projected atmospheric CO₂ concentrations in
264 2100 vary between 393 and 1135 ppm depending upon the SSP. Global mean surface air temperatures
265 are projected to rise between 1.1 and 5.8 °C (mean of scenarios) compared with the 1750 baseline,
266 depending on the SSP. The 95th percentile temperature rise to 2100 of the most extreme emissions
267 scenario (SSP5-8.5), from runs of the MAGICC climate change model, is 8.6 °C (Meinshausen *et al.*,
268 2020).

269 We can compare carbon dioxide enrichment and soil warming experiments to these projections.
270 Ainsworth and Long (2021) review the results of Free-Air CO₂ Enrichment (FACE) experiments
271 conducted over the past three decades. All experiments raised CO₂ concentrations to between 500 and
272 600 ppm (or, equivalently, by 200 ppm above ambient), well within the range projected for 2100 by
273 SSP scenarios. Several meta-analyses of soil warming experiments have been published in recent
274 years. Unfortunately, few of these list or summarize the degree of warming applied in the original

275 studies (e.g. Meng *et al.*, 2020). In a meta-analysis of grassland carbon flux responses, experimental
276 treatments raised soil temperatures by $+1.8 \pm 1.0^\circ\text{C}$ and air temperatures by $+2.0^\circ\text{C} \pm 1.3^\circ\text{C}$ (Wang *et al.*, 2019). In studies reported by a meta-analysis of warming effects on the carbon cycle, air
277 temperatures were raised by $+1.82 \pm 0.17^\circ\text{C}$ and soil temperatures by $1.34 \pm 0.13^\circ\text{C}$ (Lu *et al.*,
278 2013). A meta-analysis of warming effects on soil microbial biomass reported variations in the degree
279 of warming among experimental methods. Heating cables raised temperatures by $+3.41 \pm 1.25^\circ\text{C}$,
280 greenhouses by $+1.84 \pm 0.27^\circ\text{C}$, infrared heaters by $+1.74 \pm 0.72^\circ\text{C}$, open top chambers by $+1.38 \pm$
281 1.05°C and curtains by $+0.74 \pm 0.30^\circ\text{C}$ (Xu & Yuan, 2017). Another meta-analysis on soil microbe
282 responses reported temperature treatments varying between 0.5 and 5.5°C (Romero-Olivares *et al.*,
283 2017). Temperature treatments were $+0.17$ – 5.52°C for soil moisture responses (Xu *et al.*, 2013), and
284 varied between $+0.1$ – 10.2°C for terrestrial ecosystem responses (Wu *et al.*, 2011).
285

286 Overall, experimental warming treatments raise soil (or air) temperatures well within the range
287 expected to 2100, as do FACE experiments with CO_2 concentrations. These are realistic treatments,
288 very different to those applied in N deposition simulations. For comparison, the median ratio between
289 experimental and background N deposition reported by Zhang *et al.* (2018) is 9.0. Applied to FACE
290 treatments, this ratio would yield an experimental CO_2 concentration of 3600 ppm. This level of
291 atmospheric CO_2 has probably not been seen on Earth since the Cretaceous Period (Royer *et al.*,
292 2004). Temperature increases of the order used in N deposition experiments would be similarly
293 extreme, and unhelpful in understanding the implications for future climate change.

294 **Learning from N deposition gradients**

295 High experimental N deposition treatments may be chosen in the hope of eliciting an ecosystem
296 response during the short timeframes usually available for funded academic research. However,
297 comparison of cumulative dose-response curves demonstrates that ecosystem responses can be
298 fundamentally different under low and high N deposition rates. For example, tree growth rates were
299 much lower under high compared with low N experimental addition rates, for the same total
300 deposition (Binkley & Högberg, 2016). Hence, there is no short cut to understanding N deposition
301 effects via unrealistic experimental treatments.

302 Correlative studies utilizing existing N deposition gradients offer an alternative approach. Analysis of
303 growth and survival rates of nearly 1.5 million trees in 94 species across the USA revealed a diversity
304 responses to N deposition gradients (Horn *et al.*, 2018). The minimum N deposition rate experienced
305 per tree species ranged from 0.9 to 7.2 (mean 3.6) $\text{kg ha}^{-1} \text{yr}^{-1}$ to 5.4 to 55.4 (mean 23.7) $\text{kg ha}^{-1} \text{yr}^{-1}$.
306 Of 39 species showing significant growth responses, 20 had increasing growth rates, while 17 species
307 had humped responses peaking between 6 and $21 \text{ kg N ha}^{-1} \text{yr}^{-1}$. Only 2 species had decreasing growth
308 with N deposition. Far more species had humped responses for survival, than had linear responses.
309 Responses of forest understorey plant communities to N deposition are dependent on the

310 environmental context (Perring *et al.*, 2018). Analysis of 1814 plots across Europe, spanning a
311 deposition gradient of around 7 – 30 kg N ha⁻¹ yr⁻¹, showed that cover of graminoids tended to
312 increase with N deposition for low light communities and decrease with N for high light communities.
313 Cover change for forbs peaked around 17 kg N ha⁻¹ yr⁻¹ but decreased at higher and lower deposition
314 levels.

315 Fungal responses to N deposition gradients have been assessed in a number of studies across Europe
316 and the USA (Supplementary Table S1). The majority of research has been conducted in forests,
317 along with two studies in bog habitat and one in grassland. The minimum N deposition rate among
318 studies varied between 0.8 and 16.8 (mean 7.0 kg N ha⁻¹ yr⁻¹), and the maximum between 6.7 and 46.0
319 (mean 22.6) kg N ha⁻¹ yr⁻¹. No significant effect has been detected on soil fungal biomass (Nilsson *et al.*
320 *et al.*, 2007; Moore *et al.*, 2021) nor on the fungal:bacterial biomass ratio (Liu & Crowley, 2009).

321 However, reduced growth rates and biomass were detected under increasing N deposition for both
322 ectomycorrhizal fungi (Nilsson *et al.*, 2007; Ostonen *et al.*, 2011; Kjølner *et al.*, 2012; Bahr *et al.*,
323 2013; de Witte *et al.*, 2017) and arbuscular mycorrhizal fungi (Nilsson *et al.*, 2007). Mycorrhizal
324 fungal diversity and species richness tends to decline with increasing N deposition (Lilleskov *et al.*,
325 2002; Suz *et al.*, 2014; de Witte *et al.*, 2017; Ceulemans *et al.*, 2019; van Geel *et al.*, 2020), and shifts
326 in fungal community composition along N deposition gradients have been detected (Lilleskov *et al.*,
327 2002, 2008; Jarvis *et al.*, 2013; Andrew *et al.*, 2018; Moore *et al.*, 2021). There are few studies on
328 litter decomposition along N deposition gradients, a process that is strongly determined by fungal
329 activity. Contrasting effects on litter decomposition rates were detected along forest edge deposition
330 gradients, with a decrease in deposition with N in Corsican pine forest but increase in beech forest
331 (Vanguelova & Pitman, 2019). Peat decomposition increases with N deposition (Bragazza *et al.*,
332 2006), but litter layer enzyme activity was not found to vary with N across a large-scale European
333 study (Andersson *et al.*, 2004). In summary, there appears sufficient evidence to suggest that
334 mycorrhizal fungi are negatively affected by N deposition, while the response of saprotrophs remains
335 unclear.

336 Observational studies across N deposition gradients offer another advantage over those experimental
337 studies that employ one or two high treatment rates, namely, the potential to fit response functions to
338 N deposition rates. The importance of this is demonstrated by the fact that a large fraction of tree
339 species in the USA show humped responses of growth and survival to N deposition, which require
340 appropriate statistical models and even coverage of the sample space to detect (Horn *et al.*, 2018).
341 Unrealistically high experimental treatments would sample only the extremes this response, missing
342 the ecologically relevant curve altogether (Fig. 6). Correctly defining the shape and scale of
343 ecosystem responses to N deposition is key to models of carbon and N cycling, and to understanding
344 how the biosphere will respond to global change (Davies-Barnard *et al.*, 2020; Wiltshire *et al.*, 2020).
345 A disadvantage, shared with all observational studies and natural experiments, is that causation cannot

346 strictly be proven. Great care is required to gather data on possible confounding factors and to sample
347 in such a way that correlations among predictors are minimized, or collinearity will prevent the effects
348 of different predictors from being disentangled (Breeuwer *et al.*, 2008).

349 **Conclusions**

350 N is a major limiting nutrient and an important determinant of ecosystem productivity and function.
351 Anthropogenic activities have dramatically altered the global carbon cycle, through application of
352 mineral fertilizers in agriculture, and through atmospheric N deposition following biomass and fossil
353 fuel combustion. Understanding the impacts of anthropogenic N has been a major goal of global
354 change research, through observational and experimental studies. However, there remains a wide gulf
355 between global N deposition rates, and the experimental treatments applied to simulate them. The N
356 treatments applied in thousands of experiments reported in hundreds of studies and summarized in
357 numerous meta-analyses and reviews are around an order of magnitude larger background deposition
358 rates, and are similar to mineral fertilizer applications in agricultural ecosystems. Where the purpose
359 of an experiment is to understand the effects of agricultural fertilizers, then high levels of N
360 application rates are justified. Unfortunately, many decades of experimental work have left us with a
361 poor understanding of biological responses to anthropogenic N deposition. Modelling biosphere
362 responses to global change could be hampered or biased by this knowledge gap. The responses of
363 organisms to enhanced N availability can be subtle and non-linear, and effects on natural ecosystems
364 may be better understood using spatial deposition gradients or more realistic experimental treatments.

365

366 Data and Methods

367 ISIMIP2b N deposition data used in Figs. 1, 2 and S1 were obtained from [https://esg.pik-](https://esg.pik-potsdam.de/projects/isimip/)
368 [potsdam.de/projects/isimip/](https://esg.pik-potsdam.de/projects/isimip/)

369 Further information on ISIMIP2b data are available from [https://www.isimip.org/gettingstarted/input-](https://www.isimip.org/gettingstarted/input-data-bias-correction/details/24/)
370 [data-bias-correction/details/24/](https://www.isimip.org/gettingstarted/input-data-bias-correction/details/24/)

371 Data on N deposition rates reported in meta-analyses and reviews (Figs. 3, 4 and 5) were obtained
372 from supplementary information published with these sources. No processing was conducted on these
373 data other than conversion of units where required, and data are reported ‘as is’.

374 Plots were generated using package *ggplot2* v.3.3.2 for R v. 4.0.3. Package *mgcv* v. 1.8–33 supplied
375 the Generalized Additive Models (GAMs) used to produce smooth interpolations in Fig. 4.

376 Weightings used for the GAMs were calculated according to Lajeunesse (2015).

377

378 Conflict of interest statement

379 The author declares no conflict of interest.

380

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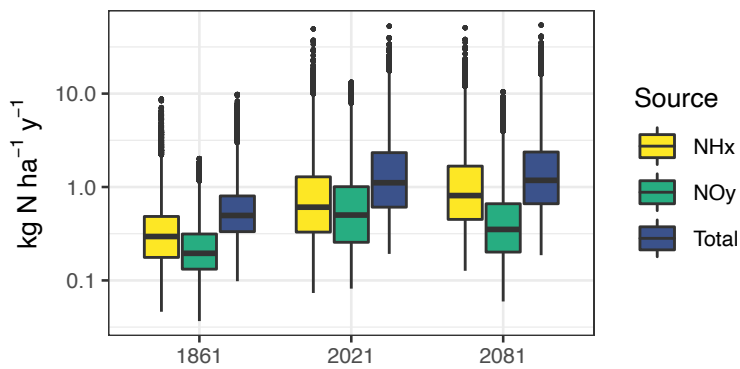
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668 **Figures**

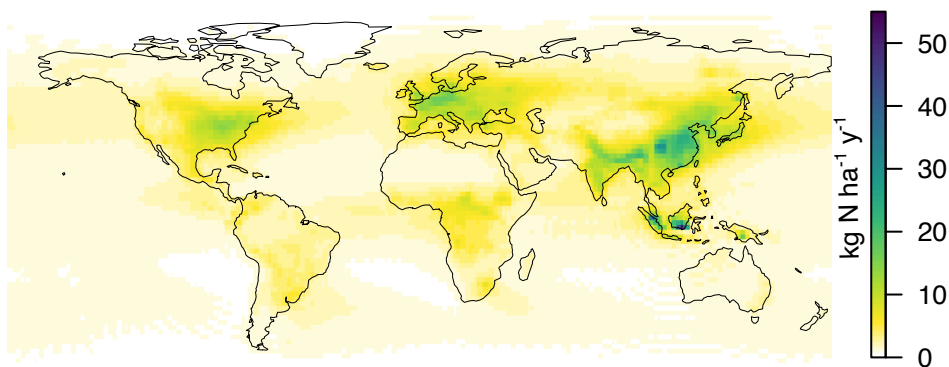
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671 **Fig. 1. Land surface N deposition rates in ISIMIP2b simulations at 0.5° resolution, for pre-**
672 **industrial (1861), current (2021) and future (2081) years.** Both current and future rates are
673 modelled using the RCP6.0 emissions scenario. Boxes show interquartile range, bars show medians,
674 for pixel counts uncorrected for area.

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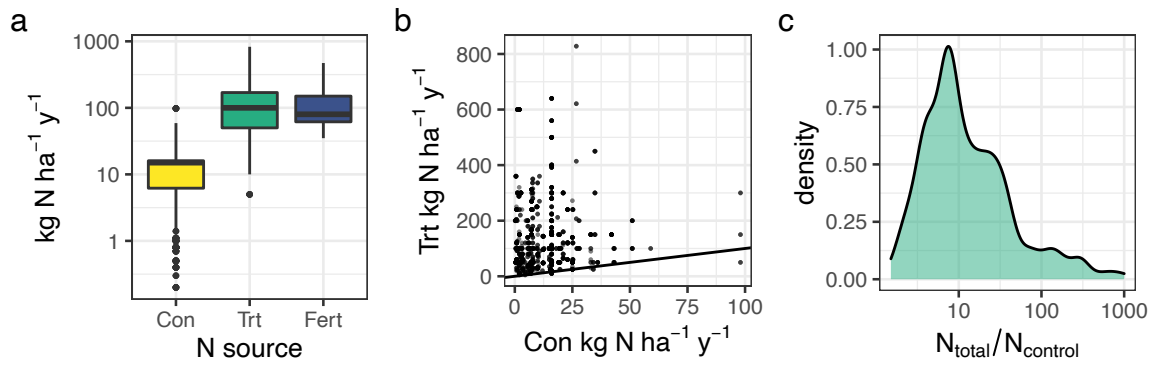
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677 **Fig. 2. Total N deposition rates in ISIMIP2b simulations at 0.5° resolution for year 2021.**

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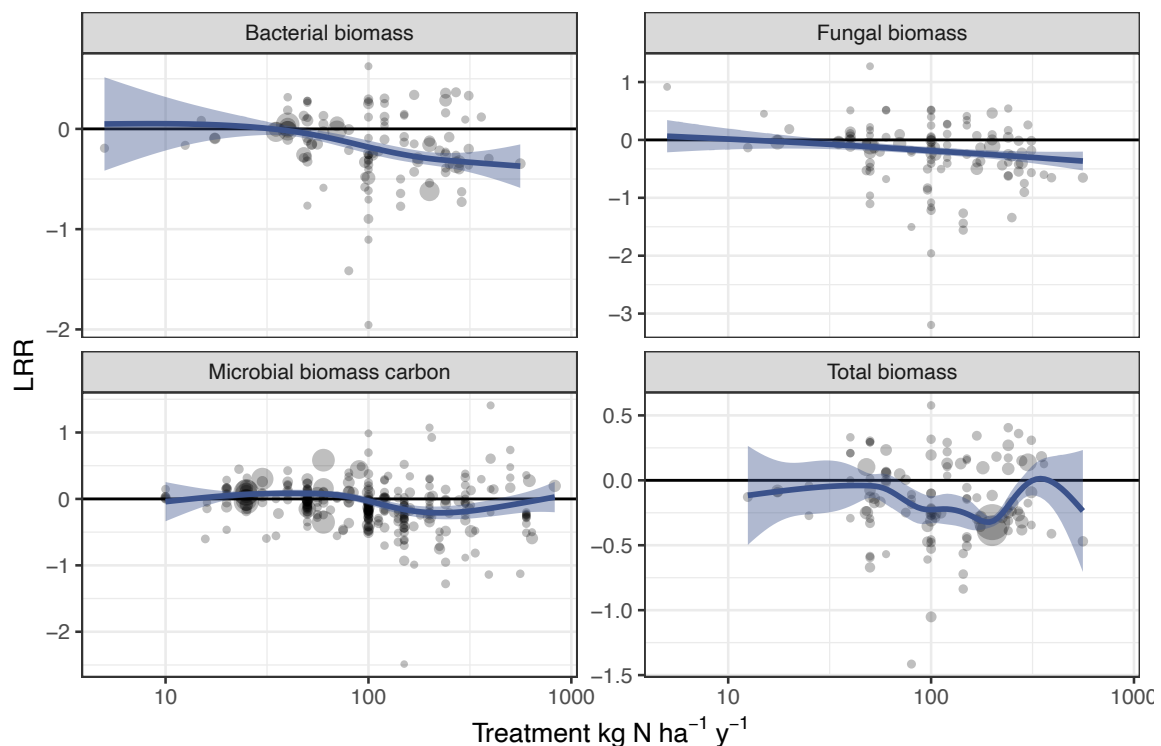
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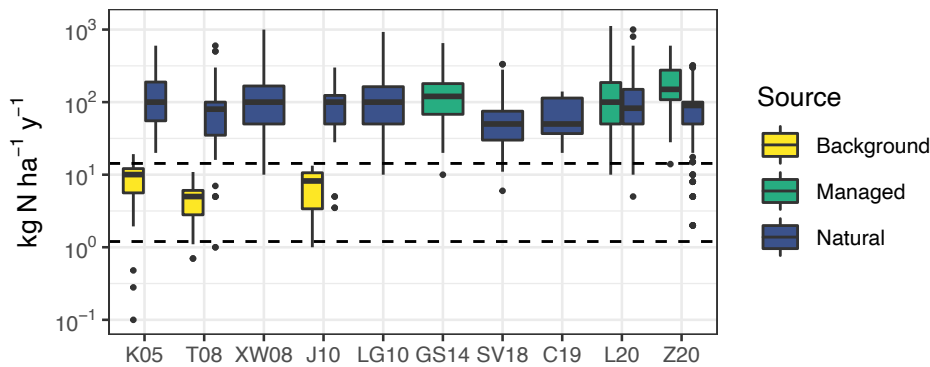
682 **Fig. 3. Experimental N deposition from a recent meta-analysis.** a) N deposition rates in
 683 background (Con) and treatment (Trt) samples reported by Zhang et al. (2018). N additions from NPK
 684 fertilizer treatments (Fert) from a separate meta-analysis are shown for comparison (Bebber &
 685 Richards, 2020). Note log scale for N deposition rates. b) Pairwise comparison of N deposition rates
 686 in treatment and controls reported by Zhang et al. (2018). Black diagonal shows doubling of N
 687 deposition (treatment = background rate). c) Density plot for ratio of total N deposition (background +
 688 experimental addition) to control (background) reported by Zhang et al. (2018).

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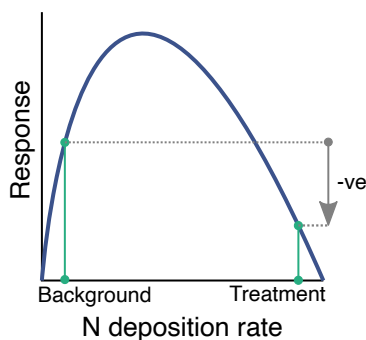
691 **Fig. 4. Log response ratio vs. treatment N deposition for four variables reported in Zhang et al.**
 692 **(2018).** Bold lines and shaded areas show means and 95% confidence limits for GAMs weighted by
 693 inverse variance and fitted to log-transformed experimental N deposition. Size of point is indicative of
 694 relative weight for each comparison.



695

696 **Fig. 5. N deposition rates in meta-analyses and reviews.** Data sources are K05 (Knorr *et al.*, 2005),
 697 T08 (Treseder, 2008), XW08 (Xia & Wan, 2008), J10 (Janssens *et al.*, 2010), LG10 (Liu & Greaver,
 698 2010) and GS14 (Geisseler & Scow, 2014), SV19 (Schulte-Uebbing & de Vries, 2018), C19 (Cheng
 699 *et al.*, 2019), L20 (Liang *et al.*, 2020), Z20 (Zhou *et al.*, 2020). Boxes show interquartile range, bars
 700 show medians. Lower and upper dashed lines show median ($1.2 \text{ kg N ha}^{-1} \text{ y}^{-1}$) and 99th percentile
 701 ($14.2 \text{ kg N ha}^{-1} \text{ y}^{-1}$) global deposition rates for 2021 in the ISIMIP2b simulation. N deposition
 702 treatments in natural ecosystems (blue) are similar to agricultural fertilization rates (green) and are
 703 around an order of magnitude larger than background rates (yellow). Background deposition in
 704 Janssens *et al.* (2010) is wet deposition only. A single data point of $5185 \text{ kg N ha}^{-1} \text{ y}^{-1}$ in Treseder
 705 (2008) is not shown. Note log scale for N deposition.

706



707

708 **Fig. 6. Experimental treatments may draw misleading conclusions from nonlinear response**
 709 **functions.** In this hypothetical case, based on examples from tree growth rates and saprotrophic fungi,
 710 the ecological response is hump-shaped (blue curve). Very high N deposition treatments would lead
 711 to a conclusion of negative impacts of N on the process. Sampling across a deposition gradient, or use
 712 of multiple experimental treatments, would reveal the response function.

713

Supplementary Figures

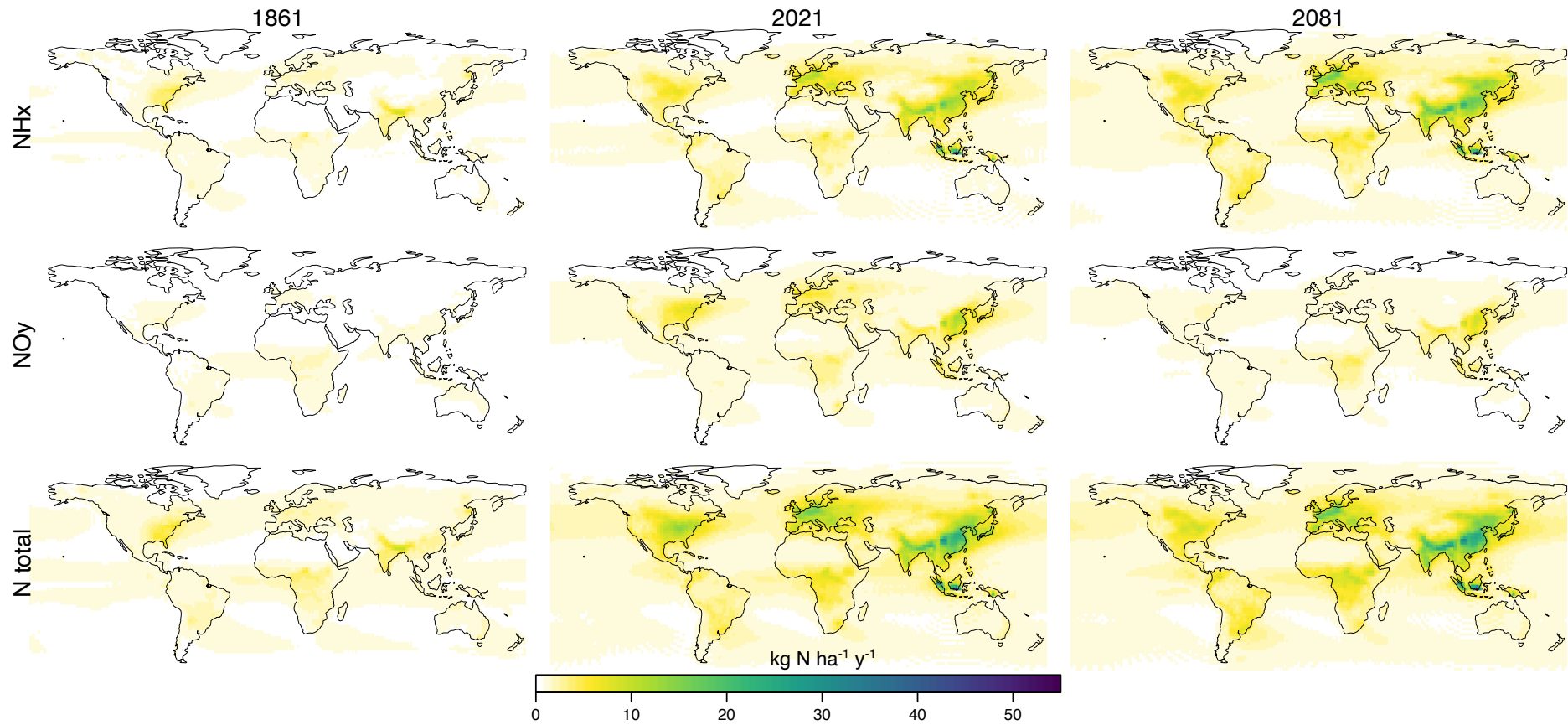


Fig. S1. Land surface N (NH_x , NO_y , total) deposition rates in ISIMIP2b simulations at 0.5° resolution for years 1861, 2021 and 2081.

Projections for 2021 and 2081 are under the RCP6.0 emissions scenario.

Table S1. Summary of N deposition gradient studies on fungi and litter decomposition published since 2000.

Study	Location	Ecosystem	N low	N high	N mean	Response variable	Direction	Notes
Van Geel et al 2020	Europe	Bog	7.4	30.5	12.6	Ericoid mycorrhizal species richness	Decrease	
Van Geel et al 2020	Europe	Heath	7.4	26.9	14.1	Ericoid mycorrhizal species richness	Not Significant	
Moore et al. 2021	Eastern USA	Forest	4.4	11.7	8.4	Soil fungal biomass	Not Significant	
Moore et al. 2021	Eastern USA	Forest	4.4	11.7	8.4	Fungal diversity	Decrease	
Moore et al. 2021	Eastern USA	Forest	4.4	11.7	8.4	Fungal community composition	Change	Community composition varied with N deposition. ECM where N deposition low, saprotrophs where N deposition high
Moore et al. 2021	Eastern USA	Forest	4.4	11.7	8.4	Fungal gene composition	Change	Oxidative enzyme genes declined with N deposition. Hydrolytic enzyme genes increased with N deposition
Ceulemans et al 2019	Europe	Grassland	6.1	21.7	14.1	Arbuscular mycorrhizal species richness	Decrease	Calcareous grassland
Ceulemans et al 2019	Europe	Grassland	4.1	31	15.1	Arbuscular mycorrhizal species richness	Decrease	Acidic grassland
Andrew et al 2018	Europe	Various				Macrofungal fruiting body assemblages	Change	
de Witte et al 2017	Switzerland	Forest	16.8	33	24.6	Ectomycorrhizal diversity	Decrease	
de Witte et al 2017	Switzerland	Forest	16.8	33	24.6	Ectomycorrhizal biomass	Decrease	
Suz et al 2014	Europe	Forest	5.1	35.5	14.5	Ectomycorrhizal species richness	Decrease	
Suz et al 2014	Europe	Forest	5.1	35.5	14.5	Ectomycorrhizal evenness	Decrease	
Jarvis et al 2013	Scotland	Forest	3.1	9.9	4.6	Ectomycorrhizal community composition	Change	
Bahr et al. 2013	Sweden	Forest	0.95	24.6	6.2	Ectomycorrhizal biomass	Not Significant	Ergosterol
Bahr et al. 2013	Sweden	Forest	0.95	24.6	6.2	Ectomycorrhizal growth	Decrease	Visual assessment
Evju & Bruteig 2013	Norway	Forest	1.5	6.7	3.4	Lichen community composition	Not Significant	
Kjøller et al 2012	Denmark	Forest	27	43	35.9	Ectomycorrhizal root tip abundance	Decrease	Edge effect
Kjøller et al 2012	Denmark	Forest	27	43	35.9	Ectomycorrhizal growth	Decrease	Edge effect
Ostonen et al 2011	N Europe	Forest	1	12.5	7.6	Ectomycorrhizal biomass	Decrease	
Liu & Crowley 2009	California, USA	Scrub				Fungi:Bacteria ratio	Not Significant	Natural gradient result only
Lilleskov et al. 2008	NE USA	Forest	2.8	7.9	5.1	Ectomycorrhizal community composition	Change	Wet deposition only
Nilsson et al. 2007	Sweden	Forest	9.5	18.3	13.8	Ectomycorrhizal growth	Decrease	Mean N deposition from site means
Nilsson et al. 2007	Sweden	Forest	9.5	18.3	13.8	Soil fungal biomass	Not Significant	Mean N deposition from site means
Nilsson et al. 2007	Sweden	Forest	9.5	18.3	13.8	Arbuscular mycorrhizal biomass	Decrease	Mean N deposition from site means
Sigüenza et al. 2006	California, USA	Scrub				Arbuscular mycorrhizal colonization	Unclear	Results are difficult to interpret
Lilleskov et al. 2002	Alaska, USA	Forest	0.9	13.8	6.8	Ectomycorrhizal species richness	Decrease	
Lilleskov et al. 2002	Alaska, USA	Forest	0.9	13.8	6.8	Ectomycorrhizal community composition	Change	
Vanguelova & Pitman 2019	UK	Forest	22	46	34	Litter decomposition	Decrease	Corsican pine forest edge
Vanguelova & Pitman 2019	UK	Forest	22	36	29	Litter decomposition	Increase	Beech forest edge
Bragazza et al. 2006	Europe	Bog	0.8	20	6.3	Peat decomposition	Increase	
Andersson et al. 2004	Europe	Forest	2.7	26.8	10.4	Litter layer cellulase activity	Not Significant	
Andersson et al. 2004	Europe	Forest	2.7	26.8	10.4	Litter layer chitinase activity	Not Significant	Chitinase correlated with ergosterol