1   **Short title: Nitrogen deposition integrated synthesis**

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3   **Title: Nitrogen deposition and climate: an integrated synthesis**

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12

13   **Abstract**

14   **Human activities have more than doubled reactive nitrogen deposited in ecosystems, perturbing the**

15   **nitrogen cycle and causing considerable impacts to plant, animal, and microbial communities.**

16   **However, biotic responses to nitrogen deposition can vary widely depending on factors including**

17   **local climate and soils, limiting our ability to predict ecosystem responses. We synthesize reported**

18   **impacts of elevated nitrogen on grasslands and draw upon evidence from the globally distributed**

19   **Nutrient Network experiment (NutNet) to provide insight into causes of variation and their relative**

20   **importance across scales. This synthesis highlights that climate and elevated nitrogen frequently**

21   **interact, modifying biotic responses to nitrogen. It also demonstrates the importance of edaphic**

22   **context and widespread interactions with other limiting nutrients in controlling biotic responses to**

23   **nitrogen deposition.**

24

25

Reactive nitrogen and biotic responses

Reactive nitrogen (N) impacts human health [1] and shapes the diversity, composition, and function of the biosphere [2-4]. Yet, in less than a century, human activities have more than doubled the reactive nitrogen supplied to Earth's ecosystems via atmospheric deposition [5], with some regions currently receiving more than 50 kg N ha^{-1} y^{-1} [6]. The supply of reactive N deposition (N_{dep}) on Earth is regionally variable because it is associated with agriculture and fossil fuel emissions and can be transported long distances via weather systems [6, 7].

Policies recognizing the threats of anthropogenic N to human health and ecosystem functioning have focused on regulating oxidized nitrogen, or NO_x, emissions, inducing new regional variability in N_{dep} trends. For example, N_{dep} is increasing rapidly in some regions including East Asia & Brazil [7, 8], while declining due to regulations in the United States and many European Union countries. Nonetheless, although N_{dep} in the US, for example, declined following successes of the Clean Air Act, it remains 5-10 times higher than pre-industrial levels [8], and N_{dep} across the US increased by 8% between 1984-2016, even with strong regulatory policies [6]. However, while some regional emissions have declined directly in response to NO_x regulation and indirectly via regulation of CO_2 [9, 10], less regulated N forms (e.g., reduced N, or NH_x) have come to dominate N_{dep}, with some regions experiencing substantial net increases [6, 11, 12].

From the perspective of Earth's biota, N_{dep} is occurring in the context of many ongoing changes to ecosystems, including changing climate and atmospheric CO_2. Because biotic responses to N_{dep} can vary widely depending on abiotic factors such as temperature and precipitation, our ability to predict the effects of N supply on diversity and functioning from reviews of empirical studies remains a key challenge [13]. Further, while synthesis and reviews of studies performed in different locations provide a powerful basis for summarizing published work, these approaches are limited in their ability to capture sources of variation in biotic responses across edaphic and climatic gradients because of differences in

52 methods and heterogeneous reporting of environmental conditions among studies. Reviews of N effects

53 on biota also typically combine differing N sources, although oxidized and reduced N forms can differ in

54 their effects on species growing under the same conditions [14]. Thus, while existing reviews and

55 syntheses have generated important insights into the threats posed by N_{dep} in some regions and to some

56 components of the environment, this knowledge is patchy on a global basis and can provide only limited

57 insight into causes of variation in biotic responses and their relative importance across sites, continents,

58 and studies.

59

60 Here, we fill this gap with a focus on Earth's grasslands. We briefly review studies on multi-trophic

61 impacts of N_{dep} to summarize the current state of knowledge. We build from this by synthesizing the wide

62 range of biotic responses to identical, experimental N addition across climatic gradients in the long-term,

63 multi-continent Nutrient Network experiment (Box 1; NutNet - https://nutnet.org/). While N_{dep} varies

64 widely and is lower than NutNet addition rates in most locations [6], even under future scenarios [15],

65 this synthesis provides novel insights into the types of biotic responses most likely to simultaneously

66 depend upon N inputs and climatic conditions. Using this approach, we fill knowledge gaps about N

67 impacts on grassland populations, communities, and ecosystem processes, including in the context of

68 climate, and we point to future opportunities for understanding N impacts to support ongoing

69 development of environmental standards (Box 2).

70

71 **Plant diversity and species traits**

72 One of the most widely reported responses to N_{dep} is the loss of species from plant communities, with

73 abundance declines and local extinction even at low rates of N input [14, 16, 17]. Across experiments,

74 climate can determine species loss rates which can continue for years [14]. While individual species'

75 responses to N_{dep} vary among sites and studies [8, 16], compositional changes frequently result in reduced

76 species richness [17, 18]. N_{dep} can favor grasses over forbs, with shorter-statured, low tissue N species at

77   greatest risk of local extinction [8]. Invasive species often increase with elevated N_{dep} [8]. While

78   competition for light may underlie N-induced changes in species composition and biodiversity loss [19],

79   soil acidification can exacerbate biodiversity loss in unbuffered soils [18]. Long-term simulated N_{dep} can

80   even deplete seed banks, reducing the capacity for recovery [20].

81

82   *Diversity trajectories and N.* Results from the NutNet experiment (Box 1) are concordant with this

83   literature, showing that grassland plant diversity responds similarly across continents and conditions, with

84   continued losses over a decade of chronically elevated N [21]. These directly comparable data further

85   demonstrate that this diversity loss trajectory is exacerbated by high background rates of other soil

86   elements [22] and is compounded when other elements, like phosphorus that arrives in many locations via

87   long-distance transport [23], are also added. These results corroborate consistent N impacts on grassland

88   plant diversity across conditions, and advance understanding by demonstrating that the magnitude of N

89   impacts on diversity loss through time depend on edaphic conditions and input rates of other elements

90   (Fig. 1, Table 1). Although herbivory can maintain grassland diversity at sites where it increases ground

91   level light, herbivores do not consistently counteract the effect of elevated N on diversity [24].

92

93   *Plant trait responses to N.* Leaf N concentrations in 243 species of dominant plants in NutNet plots

94   spanning 4 continents increased with a consistent rate of N addition due to elevated within-species N

95   content [via e.g., luxury uptake, 25] and increased domination by N-rich species [26]. Non-native

96   graminoids in sites around the world also tend to have more N-rich foliage than their native counterparts

97   [27], and native abundance and richness decline while non-native dominance and richness increase with

98   N addition [28, 29], suggesting a likely trait advantage under increasing N_{dep}. This shift induced by

99   elevated N alters community trait distributions, favoring grasses and annual plants [28] and causing

100   widespread declines in legumes [30] (Fig. 1, Table 1). Plant community trait shifts induced by N supply

101   can arise from – and impact – consumer communities (see *Consumer responses*). For example, the supply

102   of nutrients including N can interact with usually unmeasured traits, like foliar sodium, to alter trait

103   abundances as a function of herbivory [31].

104

105   *Traits, N, and climate.* Whereas elevated N across NutNet sites tends to increase the local richness (m^2

106   scale) of non-native species, this effect is reversed with increasing interannual precipitation variability,

107   such that at sites with highly variable precipitation, added N tends to cause non-native species losses. In

108   contrast, native species tend to persist with N addition when interannual precipitation is variable [29].

109   Added N also reduces legume abundance, particularly at sites with high mean annual temperatures,

110   suggesting that N-deposition may be increasingly detrimental to biological N cycling with increasing

111   annual temperatures [30] (Fig. 1, Table 1).

112

113   **Plant live biomass**

114   Increased aboveground biomass is a frequently reported response to elevated N, both via N_{dep} and

115   experimental fertilization [32]. However, increasing N_{dep} shifts communities towards limitation or co-

116   limitation of biomass production by other elements, including P [33] or micronutrients [34, 35].

117   Precipitation can interact with N to constrain biomass production [36]. Plant diversity also can constrain

118   biomass production, and biomass can have a reciprocal effect on diversity [37], with chronically elevated

119   nutrient supply potentially reducing biomass gains via species extinctions over the long-term [38] and

120   reducing temporal stability [39]. Although aboveground biomass often is reported to increase with added

121   N, this is less often reported for belowground biomass, leading to reduced root:shoot ratios [40].

122   However, reviews across studies are limited in their ability to uncover climatic and edaphic interactions

123   with N that may control biomass production.

124

125   *Biomass and N_{dep}.* While most work in NutNet has focused on experimentally added N, the network also

126   has quantified impacts of N_{dep} on grassland biomass around the world. Directly comparable NutNet data

127   spanning five continents demonstrate that for every 1 kg N ha^{-1} yr^{-1}, standing plant biomass increased by

128   3%. N_{dep} was a better predictor of plant biomass than site-level climate or even plot-scale edaphic

129   properties [41].

130

131   *Nutrient effects on above- and belowground biomass.* NutNet experimental N supply increased grassland

132   aboveground biomass at only ca. 24% of sites after 3 years [42], but this effect amplified at most sites

133   through a decade [21]. However, the availability of other elements determines the strength of this

134   response [43]. When N is supplied with other elemental nutrients, peak season biomass increased rapidly

135   at ca. 66% of sites [42], and biomass at most sites increased in response to a chronic supply of multiple

136   nutrients after a decade [21], demonstrating widespread nutrient co-limitation and impacts that can take

137   years to manifest. Root allocation increased in response to N supply at grasslands spanning four

138   continents [44], but canopy density determined site effects. Root mass declined with N addition at sites

139   with dense aboveground canopies but increased at sites where canopies were sparse [44].

140

141   *Nutrient effects on diversity, production, and stability of biomass.* After a decade of chronic N addition at

142   NutNet sites spanning continents, biomass continued to increase in spite of widespread diversity loss [21].

143   N supply reduced the temporal stability of peak biomass across NutNet, acting primarily by reducing the

144   stabilizing force of interannual species asynchrony rather than via diversity loss [45, 46]. Thus, while

145   grassland diversity loss from elevated N is widespread, diversity loss is not the most likely pathway by

146   which N_{dep} will alter peak biomass and interannual variation.

147

148   *Biomass, N, and climate.* Aboveground biomass responsiveness to NutNet N addition is generally greatest

149   at higher latitude, cooler sites, with weaker responses to the same N supply rate at warmer, low latitude

150   sites [42], raising the possibility that with rising temperatures, grassland aboveground biomass will be

151   increasingly limited by factors other than N supply. NutNet studies examining drought effects have

152   focused on N addition in combination with other elements; however, these results build on previous

153   studies of N alone at single sites or in syntheses. Chronically elevated nutrients increase dominance by

154   grasses, which exacerbated impacts on biomass of a widespread European drought [47]. Across sites,

155   nutrient impacts during drought depend on site aridity and regional floras [48, 49]. Across regions,

156   elevated nutrients generally increase grassland sensitivity to precipitation variability [50] (Fig. 1, Table

157   1).

158

159   **Consumers – grassland vertebrates, arthropods, and pathogens**

160   N_{dep} may impact consumers through changes in plant quality and quantity, stoichiometric imbalances,

161   and, for arthropods and pathogens, a cooler, moister microclimate resulting from increased aboveground

162   biomass, decreased reproductive habitat, or reduced availability of host or prey species [51]. Increased

163   insect herbivory in response to elevated foliar N concentrations has been reported in some locations [52],

164   even inducing a population explosion in one documented case [53]. N_{dep} can alter nectar chemistry [54]

165   and may reduce forb richness [18], inducing pollinator declines [55, 56]. Although widespread butterfly

166   population declines in Europe have been linked to N_{dep}-related increases in vegetation productivity and

167   resulting changes in microclimates [57], most evidence demonstrating an impact of N_{dep} on arthropods

168   comes from studies of individual herbivore species [52]. Pathogen infection prevalence can increase with

169   N because N supply tends to favor faster growing, less defended plant species [58]. N_{dep} can impact

170   vertebrate consumers via reduced habitat quality and invertebrate food sources (e.g. northern flying

171   squirrel, *Glaucomys sabrinus* [59]; red backed shrike, *Lanius collurio* [60]), although extensive

172   knowledge gaps remain about N impacts on higher trophic levels [4].

173

174   *Plant quantity and quality changes with N.* N addition in NutNet alters plant traits, generally increasing

175   foliar nitrogen at the species level [26] and in aggregate biomass [61], likely improving foliage quality as

176   a food source. N_{dep} [41] and experimental N supply [42] also tend to increase aboveground grassland

177   biomass. From the perspective of plant pollinators, NutNet provides some evidence that elevated N can

178   change flowering phenology, at least in some regional floras [62]. Finally, while there are no direct tests

179   of the impact of N alone on plant defense in NutNet, an experiment performed at the same sites but with

180   the addition of multiple elements, including N, uncovered evidence for increased investment in growth

181   and reduced defense with elevated nutrients [63]. This is consistent with reduced defense in simulated

182   N_{dep} studies [52] but extends the meta-analytical result by demonstrating the consistency of this response

183   across sites and continents.

184

185   *Consumption, infection, N, and climate.* Invertebrate damage on plant foliage at nearly 30 NutNet sites

186   increased in response to N addition, with greatest damage on grasses and non-leguminous forbs [64].

187   Pathogen damage on these same plants increased with N addition on grasses and legumes [64]. Arthropod

188   and pathogen damage increase with site-level precipitation, but variation in damage with climate is

189   independent of N supply. In contrast, when plants are protected from vertebrate herbivores, the supply of

190   nutrients including N increases biomass N most strongly at low precipitation sites, but when vertebrate

191   herbivores at dry sites can access fertilized plants, they consume the nutritious biomass, reducing

192   aggregate biomass N content [61, 65]. Nutrient supply also interacts with foliage chemistry and herbivory

193   across this climate gradient. In particular, foliage at sites with low precipitation is relatively high in

194   sodium – a critical element for consumers – and under dry conditions, vertebrate herbivores tend to

195   consume foliage of high sodium plant species in fertilized plots [31] (Fig. 1, Table 1).

196

197   *Consumer biomass and N.* The NutNet experiment demonstrates N supply supports a greater mass of

198   arthropods (i.e., secondary production) via compositional turnover to favor arthropod species with larger

199   average body sizes [66]. Arthropod abundance also increases with N supply across these sites [66, 67],

200   particularly in response to greater mass of live and dead vegetation [66].

201

202   **Consumers – soil microbial communities**

203   While soil microbes have long been considered in the context of ecosystem processes and agriculture, the

204   edaphic and climatic characteristics controlling microbial biomass and diversity across global gradients

205 are only beginning to be uncovered [68, 69]. N addition is known to impact soil microbial communities,

206 often reducing biomass [70] and increasing dominance of bacteria over fungi [71]. Impacts of N via soil

207 acidification may be especially important in controlling species composition [72]. Nitrogen also has the

208 potential to shift the relative abundance of functional groups, with loss of e.g., mycorrhizal fungal

209 mutualists [73].

210

211 *Soil communities, functional traits, N, and climate*. NutNet data demonstrate that elevated N causes

212 predictable responses in soil microbial composition, and the magnitude of these changes tends to mirror

213 the magnitude of plant communities changes in response to N [74]. N addition tends to increase soil

214 pathogenic fungi [75] and the abundance of archaea and fungal groups like Ascomycota, whereas

215 Glomeromycota, a group that dominates the arbuscular mycorrhizal fungi, consistently decline in

216 abundance [74]. Across NutNet sites, N also reduces phosphorus solubilizing bacteria [76]. These soil

217 pathogens and mutualists do not vary with climate, but are, instead, constrained by plant composition and

218 edaphic characteristics [75] which change with N supply (Fig. 1, Table 1).

219

220 *Soil microbial biomass and N*. Grassland microbial biomass and microbial biomass C:N chemistry was

221 unchanged in response to NutNet's consistently increased N supply across a wide range of climatic and

222 edaphic conditions [77-79]. Although N did not generally reduce soil pH, N supply reduced microbial

223 genes associated with metabolism, reducing both microbial growth and respiration, particularly in surface

224 soils [78, 80].

225

226 **Ecosystem pools and rates**

227 N addition to ecosystems has considerable potential to alter processes, including cycling of N, C, and

228 other nutrients. Recent focus has been on impacts of N_{dep} on carbon cycling [81], uncovering high among-

229 study variability in the response of soil processes to N_{dep}. For example, a meta-analysis of the effect of N

230 on litter decomposition showed that responses depended on N_{dep} or N addition rate and litter quality [82].

231   While soil respiration is commonly reduced by N addition [83], impacts on soil carbon stocks are variable

232   [84]. Although some studies have found relationships between N addition and N mineralization (e.g.,

233   unimodal relationship in a tropical grassland) [85], the documented impacts on N cycling are variable in

234   the literature, apparently due to dependence of microbially-driven processes such as mineralization and

235   immobilization on climate, management, and other factors [e.g., 86].

236

237   *Litter and decomposition, N, and climate*. Although N increases live biomass in the NutNet experiment,

238   elevated N supply has variable effects on litter across sites around the world [21]. Litter disappearance is

239   poorly predicted by climate alone, instead varying most among continents, suggesting the combined

240   importance of factors including vegetation, microbial communities, and climate [87]. N accelerates initial

241   decomposition and biochemical transformation of standard substrates, particularly at cool sites, but not at

242   warm sites [88]. Further, like aboveground biomass, decomposition is co-limited by multiple nutrients,

243   and precipitation jointly determines this rate. However, while N speeds decomposition in the initial years,

244   it slows late-stage decomposition [89] (Fig. 1, Table 1).

245

246   *Soil carbon, N, and climate*. Among sites, NutNet N addition causes losses and gains of soil C, but

247   identical treatments do not induce consistent responses after 2-3 years [90] or 10 years [91]. N effects on

248   soil C likely occur where N supply reduces the soil organic C to N ratio and increases soil aggregation,

249   reducing microbial respiration and increasing soil microbial carbon use efficiency [78, 80]. The

250   availability of other elements likely plays an important role in constraining N effects on changes in soil

251   carbon [92]. When other elements are supplied along with N, changes in both soil C stocks [90] and

252   microbial carbon use efficiency [78] vary along a gradient of water (precipitation, soil sand content) (Fig.

253   1, Table 1).

254

255   *Soil nutrient cycling, N, and climate*. Across NutNet, N mineralization (N_{min}) in grassland soils is greatest

256   at sites with high microbial biomass, soil organic C, and warm, wet growing seasons [93], and elevated

257   nutrients tend to reduce N_{min} only when vertebrate herbivores are excluded from plots. Particularly under

258   elevated nutrients, the impact of herbivory on the soil N pool varies with climate [94]. While these studies

259   received N in combination with other nutrients, they demonstrate that N cycling is controlled via multiple,

260   interactive factors including elevated N under field conditions. Other work in NutNet demonstrates that

261   elevated N alone increases P cycling by increasing bacterial phosphatase production [76] (Fig. 1, Table

262   1).

263

264   **Concluding remarks**

265   Because regions around the world differ in climate, biota, and input rates of N and other elements, and

266   changes in these factors differ among regions in both direction and pace, sorting apart the sources of

267   variation in biotic responses to N_{dep} remains a difficult challenge. This is particularly true because most

268   experiments are performed at single sites and characterize one or a limited suite of response variables,

269   making it difficult to uncover site or regional contingencies. The NutNet experiment, adding an identical

270   rate of N across locations and conditions, supports understanding of the contingencies determining the

271   direction and magnitude of biotic responses. The interplay of this multi-continent experiment with the

272   existing literature on N_{dep} highlights emergent, synthetic perspectives and points to future directions (see

273   Outstanding Questions).

274

275   *Replication across time and space*. Variation in N_{dep} and its effects across both time and space mean that

276   empirical evidence for the rate, locations, and types of biotic impacts is heterogeneous. Many studies have

277   documented N limitation of biomass, but this review highlights that while N limitation is rapidly apparent

278   at a small subset of sites [42], it becomes apparent in an increasing number of locations with longer,

279   chronic inputs [21]. This review also highlights that N effects on plants, consumers, and ecosystem

280   processes vary with soil mineralogy and micronutrient supply, underscoring the need for increased

281   attention to the role of inputs of other elements [e.g., 95] and local edaphic characteristics in controlling

282   biotic responses to N_{dep}. Effects consistent across conditions raise additional questions. For example,

283 while N_{dep} increases plant palatability and reduces defense across a wide range of site conditions,

284 successful use of grazing in management will rely on understanding the condition-dependence of

285 response times [96], tipping points [97], and the role of local edaphic and climate conditions in recovery

286 potential [98]. Results from NutNet and the many other studies reviewed here suggest that long-term,

287 cross-continent experiments are needed to uncover N_{dep} impacts that may take years to emerge and may

288 differ across conditions [99]. Finally, exceedingly few experiments impose gradients of N addition [14],

289 but these will provide key insights about the shape of biotic responses and risk of tipping points with

290 increasing N_{dep}.

291

292 *Climate and N_{dep}*. While often studied in isolation, changing climate and N_{dep} rates can interact to impact a

293 wide range of biotic responses (Fig. 1, Table 1). Precipitation mean and variation are particularly

294 prominent in modifying the biotic responses reviewed here. For example, in response to NutNet's

295 elevated N supply, control of grassland biomass and plant chemistry shifts from herbivores to nutrients

296 with increasing precipitation [61, 65], whereas precipitation variability determines whether native or non-

297 native plants decline [29]. Together with scant, but compelling, evidence in the literature about impacts of

298 N_{dep} on consumers, this review also demonstrates the importance of climate in mediating N impacts with

299 potentially far-reaching, but mostly unknown, implications spanning trophic levels. The results reviewed

300 here emphasize the need to understand the dependence of biotic responses to N_{dep} on climate to avoid

301 unexpected outcomes under future conditions.

302

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307

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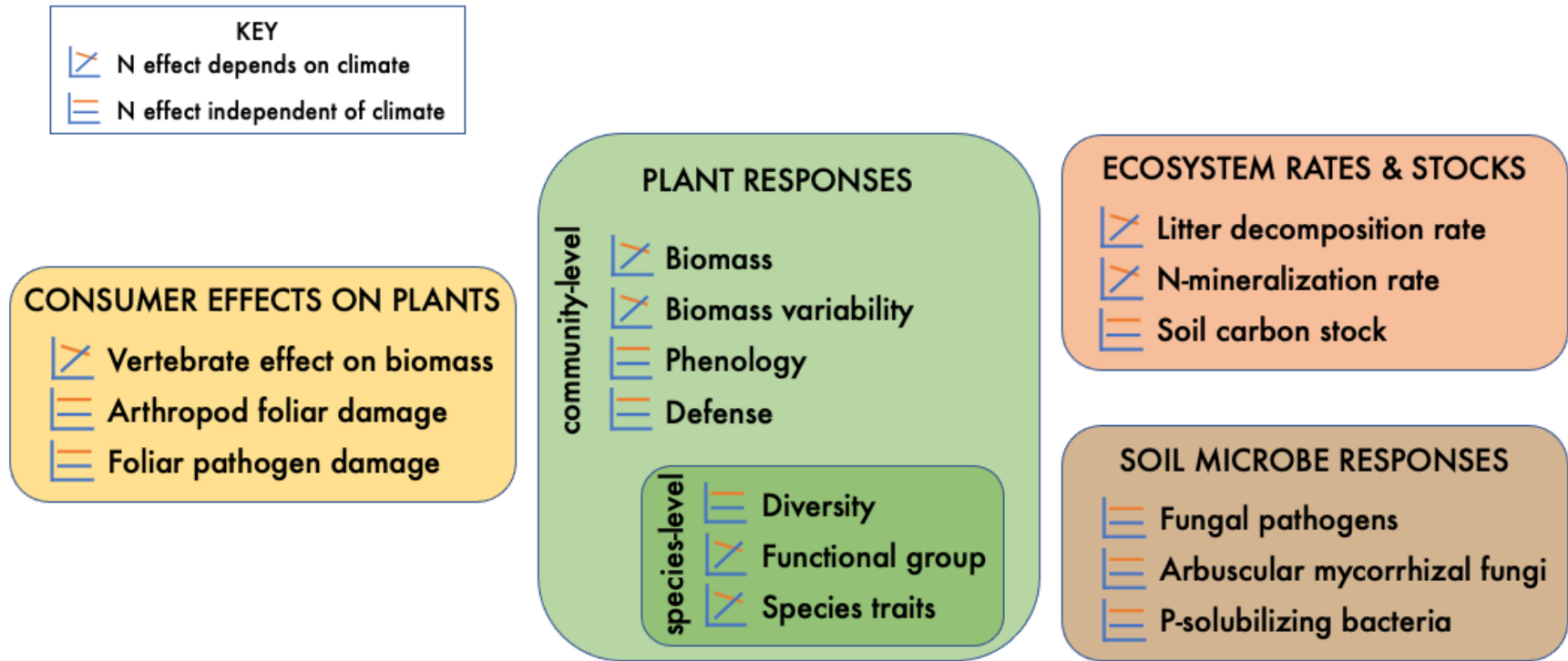
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553

554

555 **Table 1. Nitrogen and climate impacts in the Nutrient Network experiment.** In the Nutrient Network experiment, climate modifies

556 many of the biotic responses to experimental nitrogen supply. Response variables and colors relate to Figure 1.

Response Variable	N effect direction	Climate interaction with N	Citation
Plant diversity	↓		[21, 22]
Foliar nitrogen	↑	Herbivores reduce plant community foliar N at low precipitation sites	[26, 61]
Plant provenance (non-native species)	↑	Precipitation variability increases native plant species despite elevated N	[28, 29]
Functional group (legumes)	↓	Elevated temperature increases legume loss with N	[30]
Functional group (grasses, annuals)	↑		[28]
Plant biomass	↑ most consistently with addition of other nutrients	Strongest effect of N at high latitude, cool sites	[21, 42]
Plant biomass variability	↑	Stability is further reduced with drought and aridity	[47-49]
Plant community phenology	N can change community phenology in some regions		[62]
Plant community defense	↓		[63]

Arthropod foliar damage	↑	Damage increases with precipitation, but this is independent of N addition	[64]
Vertebrate impacts on plant biomass	↑	Large herbivores most strongly reduce N-fertilized biomass at low precipitation sites	[31, 61, 65]
Foliar pathogen damage	↑	Infection increases with site-level precipitation independent of N supply	[64]
Soil pathogenic fungi diversity	↑		[75]
Arbuscular mycorrhizal fungi	↓		[74]
P-solubilizing bacteria	↓		[76]
Soil microbial growth	↓		[77-79]
Soil microbial respiration	↓		[78, 80]
Litter decomposition	↑ early-, ↓ late-stage decomp.	Likely interacts with climate and other factors (vegetation, microbial community)	[21, 87, 89]
N-mineralization		Highest at sites with a warm, wet growing season	[93]
Soil carbon stock	losses and gains across sites	Varies with precipitation, particularly when other elements are added in addition to N	[90, 91]

557

KEY
- N effect depends on climate
- N effect independent of climate

CONSUMER EFFECTS ON PLANTS
- Vertebrate effect on biomass
- Arthropod foliar damage
- Foliar pathogen damage

PLANT RESPONSES

community-level
- Biomass
- Biomass variability
- Phenology
- Defense

species-level
- Diversity
- Functional group
- Species traits

ECOSYSTEM RATES & STOCKS
- Litter decomposition rate
- N-mineralization rate
- Soil carbon stock

SOIL MICROBE RESPONSES
- Fungal pathogens
- Arbuscular mycorrhizal fungi
- P-solubilizing bacteria

558

559

560   **Figure 1. Many biotic responses to elevated nitrogen depend on climate.** In the Nutrient Network experiment replicated across

561   global climates, nitrogen supply affects biotic responses, and climate modifies some, but not all, biotic responses to elevated nitrogen

562   supply. Icons indicate whether climate modifies a response to nitrogen; they do not imply magnitude or direction of effect. See Table

563   1 and text for effect direction and additional details.

Box 1: The Nutrient Network

The Nutrient Network project (NutNet), a global change study begun in 2007, is currently replicated at 152 grassland sites in 29 countries (Figure I), spanning Earth's climates. The NutNet collaboration is generating three interoperable
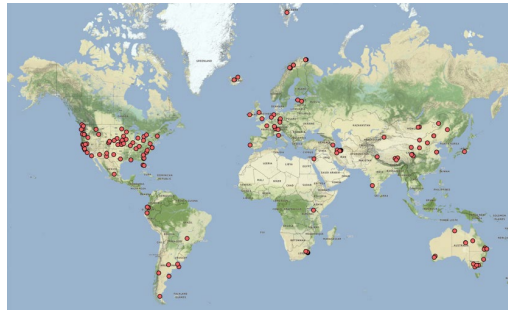

Figure I. Nutrient Network site locations (a) represent a globally relevant range of (b) mean annual temperature, (c) mean annual precipitation, and (d) nitrogen deposition rates.

datasets [100]. (**1**) *Observational data*. Using identical methods at all sites, this project is generating a spatially extensive dataset on grassland biodiversity and ecosystem characteristics. (**2**) *Multi-nutrient experiment*. At >100 sites, NutNet scientists are applying a factorial combination of nitrogen (N: 10 g N $m^{-2}y^{-1}$ as slow-release urea, CH_4N_2O), phosphorus (P: 10 g P $m^{-2}y^{-1}$ as $Ca(H_2PO_4)_2$), and potassium (K: 10 g K $m^{-2}y^{-1}$ as K_2SO_4) with micronutrients (1-time addition in K treatment plots of a micronutrient mix, μ, containing Ca, Mg, S, B, Cu, Fe, Mn, Mo, and Zn). (**3**) *Nutrient and herbivory experiment*. At >100 sites, NutNet scientists are applying a factorial combination of large herbivore exclusion (via fences) and annual NPKμ addition.

At all NutNet sites, annual data on grassland plant species identity, cover, biomass, and light interception, have been collected from a median of 30 plots per site using identical sampling protocols. Soil chemistry and texture data also have been collected from every plot around the world at regular intervals. Many sites have collaborated on collection of additional response data, including arthropods, foliar chemistry and damage, and soil microbial composition and functioning. Slow-release urea, released into the environment as a function of site-level precipitation, is an effective proxy for N deposition [101], and the experimental N supply rate, while high, is less than double some currently observed regional deposition

589 rates [6]. High experimental addition rates are often used as a proxy for chronic, multi-decadal nutrient

590 inputs by pollution. Thus, NutNet's factorial experimental design provides directly comparable, integrated

591 data on a wide range of biotic responses to identical inputs of a single form of nitrogen (CH_4N_2O) across a

592 wide range of global climate regimes.

593 **Box 2: Standards for the control of nitrogen emissions and deposition**

594 Following the United Nations Conference on the Human Environment the Convention on Long-Range

595 Transboundary Air Pollution (CLRTAP) was signed in Geneva in 1979. This was the first multilateral

596 convention addressing air pollution, signed by 51 primarily European parties, and supported by several

597 protocols including the Gothenburg Protocol, focused on reducing acidifying pollutants, including

598 nitrogen. The CLRTAP introduced the critical loads concept, defined as "a quantitative estimate of an

599 exposure to one or more pollutants below which significant harmful effects on specified sensitive

600 elements of the environment do not occur according to present knowledge" [102]. Empirical critical loads

601 are based on evidence from studies [103] and are subject to routine review.

602

603 The United States has National Ambient Air Quality Standards for NO_x introduced in 1971 and is a

604 signatory of the CLRTAP. National critical loads for N_{dep} into ecosystems were published in 2011 [104].

605 Due to lower levels of background N deposition in the US, critical loads are often lower than European

606 equivalents and may be accompanied by lower protective target loads.

607

608 China is one of the dominant emitters of reactive nitrogen. In China, N_{dep} peaked around the year 2000

609 and declined dramatically between 2010 and 2018, as NO_x emission controls came into effect [105].

610 Critical loads in China are largely based on the steady state mass balance approach [106], although

611 empirical evidence that could be used to create empirical critical loads is growing [107], .

612

613   In other regions of the world, there is increasing awareness of the need to control nitrogen emissions, but

614   this must be balanced with food security. For example, regions in Africa and Latin America have

615   considerable shortages of agricultural nitrogen while other regions are experiencing unwanted impacts of

616   nitrogen deposition [108, 109].