

1 **Research progress on the impact of nitrogen deposition on global grasslands**

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3 Running title: Nitrogen deposition and grasslands

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21 **Highlights**

- 22 • Grasslands in many regions of the world have been impacted by atmospheric nitrogen  
23 deposition
- 24 • Nitrogen deposition commonly leads to reductions in species richness
- 25 • Increases in biomass production is a common response to increased N deposition
- 26 • In some parts of the world there has been very little research into the impacts of nitrogen  
27 deposition

28

29 **Abstract**

30 Grasslands are a very important ecosystem globally providing critical ecosystem services. The species  
31 composition and characteristics of grasslands vary considerably across the planet with a wide variety  
32 of different grasslands found. However, in many regions grasslands have been impacted by  
33 atmospheric nitrogen (N) deposition originating from anthropogenic activities with effects on  
34 productivity, species composition and diversity widely reported. Impacts vary across grassland  
35 habitats but many show declines in species richness and increases in biomass production related to  
36 soil eutrophication and acidification. At a continental level there is considerable variation in the  
37 research effort that has been put into understanding the impacts of nitrogen deposition. In Europe,  
38 North America and parts of Asia, although there are outstanding research questions, there is a good  
39 understanding of N deposition impacts in most grassland habitats. This is not the case in other  
40 regions with big knowledge gaps in some parts of the world. In this paper we review the impacts of  
41 N deposition on grasslands around the world, highlighting recent advances and areas where  
42 research is still needed.

43

44 **Keywords**

45 Acidification, biomass production, critical load, eutrophication, species composition, species richness

46

47 **1. Introduction**

48 Grasslands cover approximately 40 % of the earth's land surface (Reynolds, 2005). Globally  
49 grasslands account for 69% of the world's agricultural area (Mara, 2005), they store about 343 Gt  
50 carbon, 50 % more than the amount stored in forests globally (Conant, 2010), and are responsible  
51 for providing a host of other ecosystem services including food production, water regulation, carbon  
52 storage and recreation. They also provide us with high biodiversity of flora and fauna.

53 Globally, nitrogen (N) deposition exceeds 90 Tg N yr<sup>-1</sup> (Ackerman et al., 2019; Figure 1) which has led  
54 to a wide range of impacts in grasslands. There are two main methods used to investigate the  
55 impacts of N deposition on grasslands. The first is experimental additions of N. This approach  
56 commonly uses a replicated plot design and offers the advantage of being a controlled experiment  
57 where confounding factors are minimised but the disadvantage that it is hard to replicate N addition  
58 as it would occur in the environment. However, many experiments use high levels of N addition to  
59 mimic longer-term responses on a short timescale. Experiments can be hard to maintain long-term

60 but fortunately there are many examples of long-term N addition experiments across the world (e.g.  
61 Clark et al., 2009; Basto et al., 2015; Hou et al., 2020). A second, increasingly common approach is to  
62 utilise gradients of N deposition within the environment, either around point sources of N emission  
63 (e.g. Pitcairn et al., 1998) or across large landscape or regional gradients (e.g. Stevens et al., 2010a).  
64 Using a gradient of N deposition can present challenges in terms of disentangling variables likely to  
65 impact on soils and vegetation but offers the advantage of demonstrating real-world impacts.

66 There are a number of different mechanisms via which N deposition can impact upon plants and  
67 soils of grasslands and other habitats (Figure 2). Primary amongst these are the soil-mediated effects  
68 of eutrophication and acidification. Eutrophication occurs when nutrient N enriches the soil, this is  
69 particularly problematic when plants are adapted to low levels of nutrients. N deposition is an  
70 important driver of plant biomass in grasslands (Stevens et al., 2015) and allows competitive species  
71 to take over at the expense of more stress-tolerant species which are unable to compete effectively  
72 for limiting resources. Light is commonly a limiting resource in enriched systems with slower growing  
73 or small stature species losing out (Hautier et al., 2009; Borer et al., 2014). Acidification is also an  
74 important mechanism in grasslands on soils that are not well buffered. Nitrogen has the potential to  
75 acidify the soil via a number of routes, and reducing pH can reduce the potential species pool  
76 (Schuster and Diekmann, 2003) able to survive and lead to increased concentrations of toxic metals  
77 such as aluminium in the soil (Andersson, 1988). At very high concentrations N, and particularly  
78 ammonium, can be toxic to plants (Britto and Kronzucker, 2002). Direct toxicity can cause leaf  
79 damage and reduced growth (Pearson and Stewart, 1993) but is quite rare and typically only occurs  
80 in the vicinity of point sources. There are also a number of indirect mechanisms via which N  
81 deposition can affect plant communities, for example, elevated N inputs can make communities  
82 more susceptible to extreme climatic conditions (Caporn et al., 2000) or pests or disease (Brunsting  
83 and Heil, 1985). Changes in species composition and species richness associated with elevated levels  
84 of N have been reported in a number of grassland ecosystems world-wide (e.g. Field et al., 2014;  
85 Simkin et al., 2016). These responses are often seen above a threshold as a critical load of N, the  
86 level of deposition below which ecosystem harm does not occur based on present knowledge, and  
87 are used to drive research, management and policy responses (Nilsson and Grennfelt, 1988).

88 This paper aims to give an overview of the impacts of N deposition to global grasslands. Grasslands  
89 will be considered in Europe, North America, South America, Asia, Africa and Australasia,  
90 highlighting the current understanding of impacts and recent research.

91

## 92 **2. Europe**

93 Europe contains a wide range of grasslands spanning climatic gradients from high elevation montane  
94 grasslands such as those found in the Alps through to Mediterranean grasslands such as those found  
95 on the Iberian Peninsula. Grasslands are found across a wide range of soil types, climates and  
96 altitudes and in all regions of Europe leading to considerable variation in species composition.  
97 Within Europe, virtually all grassland habitats are heavily impacted by human activities through  
98 grazing, mowing or other management practices and in a majority of grasslands, these activities are  
99 needed to maintain the habitat and prevent succession to woodland (Dengler et al., 2020). N  
100 deposition in Europe ranges from very low levels in the North to higher values in countries like  
101 Netherlands, Belgium and Germany (Ackerman et al., 2019). There is a long history of research into  
102 the impacts of N deposition on natural habitats in Europe. Research in this area became a particular  
103 focus following the considerable impacts of high levels of acid deposition and N deposition during  
104 the 1970s and 1980s. Since then, there has been a large body of research into the impacts of N  
105 deposition on grasslands.

106 Acidic grasslands are probably the grassland type that has received most research attention in  
107 Europe in relation to N deposition over the last two decades. Stevens et al. (2004) showed clear  
108 negative relationships between N deposition and species richness in acidic grasslands; there was a  
109 reduction of one species for every 2.5 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Further research on this habitat showed that  
110 these trends were apparent across the Atlantic region of Europe where a comparable community  
111 could be found (Stevens et al., 2010a; Damgaard et al., 2011; Wilkins et al., 2016), these gradient  
112 studies showed clear impacts of N addition on both plant communities and soils. Changes in species  
113 composition in acidic grasslands are typically an increase in graminoids and a reduction in forb  
114 species (Stevens et al., 2006; van den Berg et al., 2016). Several different analytical approaches have  
115 been used to identify vulnerable species. Soils in acidic grasslands are poorly buffered and analysis of  
116 soil chemistry and plant traits has indicated that acidification was the dominant cause of changes in  
117 UK acidic grasslands managed by grazing, rather than eutrophication (Stevens et al., 2010b).

118 Grasslands with a neutral pH present a similar picture to acidic grasslands where research has  
119 demonstrated declines in species richness associated with increasing N deposition with fewer forbs  
120 and species considered indicators of low nutrient status (Roth et al., 2017; Boch et al., 2021). The  
121 world's longest running experiment investigates the impacts of nutrients to a mesic grassland. The  
122 Park Grass experiment was initiated in 1856 by John Lawes and Joseph Gilbert in a neutral hay  
123 meadow to investigate how different fertilisers impacted yield in hay meadows (Silvertown et al.,  
124 2006). The longevity of this experiment means that it has become very useful for understanding the  
125 impacts of nutrients on a wide range of response variables. This experiment has been used to

126 understand both the impacts of N deposition on grasslands in terms of species composition and soils  
127 (Goulding et al., 1998), and to assess recovery as N deposition is reduced (Storkey et al., 2015).

128 Whilst evidence for changes in species richness is less clear in calcareous grasslands (Maskell et al.,  
129 2010; Tipping et al., 2013) there is clear evidence for a change in species composition with an  
130 increase in grass cover and a decline in forbs (van den Berg et al., 2016). A number of studies  
131 identified a decline in specialist and rare species (Van den Berg et al., 2011; Diekmann et al., 2014).  
132 In Dutch calcareous grasslands considerable changes in species composition at high N were  
133 associated with an increase in the abundance of the grass *Brachypodium pinnatum* (Bobbink and  
134 Willems, 1987). Changes in the below-ground community have also been observed (Ceulemans et  
135 al., 2019). In calcareous grasslands the well buffered soils are less likely to be susceptible to  
136 acidification. Some calcareous grasslands show signs of phosphorus (P) limitation which reduced the  
137 impact of N addition (e.g. Bonanomi et al., 2009) but this is clearly not always the case.

138 In Mediterranean grasslands water often limits plant productivity but soils are also typically nutrient  
139 poor. Compared to more temperate systems there has been considerably less research in  
140 Mediterranean grasslands but there is growing evidence of impacts on soil nutrient cycling and  
141 function, increases in primary productivity and water consumption and changes in species  
142 composition (Nair et al., 2019; Luo et al., 2020). Biological crusts are important for nutrient cycling in  
143 semi-arid systems and there is evidence that they are sensitive to N deposition with impacts on  
144 nutrient cycling and functional group composition reported (Ochoa-Hueso et al., 2013; Ochoa-Hueso  
145 et al., 2016).

146 Other grasslands types such as dune, alpine and montane, and wet grasslands have received much  
147 less research although in all of these there are experiments that demonstrate similar negative  
148 impacts of N deposition (e.g. Körner et al., 1997; Sparrius et al., 2013; Stiles et al., 2017). However,  
149 further research is needed.

150 Whilst there are grassland habitats where further information is needed, in particular to support the  
151 decision making process around critical loads (Bobbink and Hettelingh, 2011), within Europe the  
152 bigger knowledge gaps exist around changes in the N deposited. Within Europe tighter legislative  
153 control and co-benefits from carbon cutting measures have reduced N oxide emissions (Fowler et al.,  
154 2004). While ammonia emissions have remained high in many regions this has led to a change in the  
155 balance between reduced and oxidised ammonia with impacts on ecosystems likely (Stevens et al.,  
156 2011). To date relatively few experiments have investigated the differential effects of reduced and  
157 oxidised N. Furthermore, total deposition is beginning to be reduced in some areas, whilst there is a  
158 lot of variability across Europe in the extent of reductions in deposition (Sutton et al., 2011) there is

159 considerable interest in how habitats will recover from N deposition. Whilst some studies have been  
160 conducted (Stevens, 2016) there is still a need for further evidence in this area.

161

### 162 **3. North America**

163 The North American continent has a diverse array of herbaceous and grassland communities  
164 spanning almost all latitudes of the northern hemisphere. A previous summary of critical loads by  
165 Pardo et al. (2011) synthesized information on N responses for herbaceous species within eight  
166 ecoregions of North America (Omernik and Griffith, 2014). Five of these ecoregions are dominated  
167 by non-forested, shrubland, and grassland systems that make up 50% of North American land area.  
168 Here we summarize studies on grassland responses using this framework where applicable. N  
169 deposition in North America is typically lower than Europe with highest values found in the west  
170 (Ackerman et al., 2019).

171 The northernmost grasslands on the continent are the tundra and the taiga. The Tundra ecoregion is  
172 on the northern edge of the continent and contains vast grassland-like plains among mesa and  
173 mountains. The low critical load range between 1–3 kg N ha<sup>-1</sup> yr<sup>-1</sup> aims primarily to protect against  
174 changes in shrub and grass cover (Pardo et al., 2011). The Taiga consists of broadly rolling uplands  
175 and lowlands with a mix of open grasslands, peatlands, and stunted forests and has a critical load of  
176 6 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Here the main effects of N that have been observed are changes in CO<sub>2</sub> exchange,  
177 increase in foliar N, and change community composition of vascular plants (Pardo et al., 2011).

178 The Northwestern Forested Mountains ecoregion has grasslands in alpine and subalpine areas. N  
179 fertilization experiments within the sites in the alpine tundra of the Rocky Mountains showed an  
180 increasing cover of an alpine sedge species (*Carex rupestris*) with N additions greater than 3 kg N ha<sup>-1</sup>  
181 yr<sup>-1</sup> as well as an increase in soil NO<sub>3</sub> leaching once fertilization exceeded 10 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Bowman  
182 et al., 2012). Cessation of fertilization led to a decrease in the enhanced *C. rupestris* cover, but little  
183 recovery of soil processes over a decade of monitoring (Bowman et al., 2018). Pardo et al (2011) give  
184 a critical load for alpine grasslands between 4–10 kg N ha<sup>-1</sup> yr<sup>-1</sup>, however, another study in Rocky  
185 Mountain National Park estimated a similar but more sensitive critical load of N for protecting  
186 subalpine vegetation from changes to biodiversity, between 1.9–3.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> (McDonnell et al.,  
187 2014). This modelling study suggested that subalpine plant species cover in Rocky Mountain  
188 National Park has already changed by more than 10% due to N deposition over the past century and  
189 future climate change is expected to increase these changes.

190 The warmer, arid areas encompass the deserts and Mediterranean ecoregions. The North American  
191 Deserts ecoregion make up most of the western United States (USA) and northern Mexico, with  
192 variation of dominant shrubs and grasses within different desert boundaries. Critical loads range  
193 from 3–8.4 kg N ha<sup>-1</sup> yr<sup>-1</sup> for increased biomass of invasive grasses and a decrease of native forbs  
194 (Bobbink et al., 2010). Responses across desert environments have been inconsistent in these  
195 responses and often correlated with years of high precipitation (Kazanski et al., 2021). An N  
196 fertilization study on the Colorado Plateau did not affect plant diversity or the abundance of a  
197 common invasive grass, *Bromus tectorum*, in fertilization plots, but N did have a negative effect on  
198 biological soil crusts which may eventually confer changes to vegetation communities (Phillips et al.,  
199 2021). Yet in other studies in these cooler deserts, *B. tectorum* often does increase with elevated N  
200 depending on local soil properties (Belnap et al., 2016). Within the Sonoran/Chihuahuas deserts of  
201 the southern USA and northern Mexico, there have also been mixed results on the impact of N  
202 deposition on grassland communities and the spread of one of the main species of concern,  
203 buffelgrass (*Cenchrus ciliaris*). Some grass dominated communities have shown resilience to  
204 vegetation changes with N additions in the absence of disturbance (Ladwig et al., 2012), but show an  
205 increase in forb growth and diversity after fire removed grass cover and water and N additions were  
206 continued (Collins et al., 2017). Another study has shown a neutral effect on *C. ciliaris* with negative  
207 effects on native vegetation, which can still lead to the accelerated spread of the invasive grass  
208 (Lyons et al., 2013).

209 The Mediterranean California ecoregion has grasslands interspersed within coastal sage scrub, dense  
210 shrublands, and oak woodlands. Many of these grasslands are becoming increasingly invaded by  
211 exotic grasses (Minnich and Dezzani, 1998; Sandel and Dangremond, 2012). Critical loads between  
212 6–33 kg N ha<sup>-1</sup> yr<sup>-1</sup> were previously established to protect against annual grass invasion that replaces  
213 native herbs (Pardo et al., 2011). Additional research showed conversion of coastal sage scrub to  
214 exotic grasslands following a disturbance was likely to occur above 11 kg N ha<sup>-1</sup> yr<sup>-1</sup> when other  
215 exotic grasslands were nearby, and thus makes future type conversion more likely as grasses spread  
216 (Cox et al., 2014). One of the mechanisms of this may be the loss of in mycorrhizal associations as a  
217 decline in the richness of native plant species and of AMF spore morphotypes occurred at a critical  
218 load of 10–11 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Allen et al., 2016). Further research evaluating changes across  
219 deposition gradients identified a similar value of 10 kg N ha<sup>-1</sup> yr<sup>-1</sup> at which significant declines in  
220 plant richness across a steep gradient of N deposition which increased soil N availability and  
221 paralleled decreases in native forb species (Valliere et al., 2017).

222 The Great Plains ecoregion spans from central Canada through the central United States to eastern  
223 Mexico. The short-, mixed-, and tall-grass prairies are interspersed with intensive agricultural

224 activities. In the plains of southern Canada, a fertilization experiment ( $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) favoured  
225 the growth of a few species leading to a decline in richness during community assembly of previous  
226 agricultural land (Harvey and MacDougall, 2018). In the central plains of the USA, fertilization at  
227 levels of  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  led to changes in community structure of the tall-grass prairie by  
228 increasing the cover of species that typically flower in May and June and reducing the cover of those  
229 species that typically flower in September and October. This response was largely driven by the  
230 increase in invasive species (Biederman et al., 2017). These experiments have additions higher than  
231 the current critical loads range of  $5\text{--}25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Pardo et al., 2011) and new research has  
232 identified responses within this critical load range. Fertilization experiments in the northern Great  
233 Plains of the USA showed an increase in tissue N and increased cover of invasive grasses at a  $4\text{--}6 \text{ kg}$   
234  $\text{N ha}^{-1} \text{ yr}^{-1}$  for badlands sparse vegetation, and  $6\text{--}10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  within more productive grasslands  
235 (Symstad et al., 2019). In the southern Great Plains a positive biomass response for grasses *Nasella*  
236 *leucotricha* and *Schizachyrium scoparium*, was observed with deposition levels measured at  $13\text{--}18$   
237  $\text{kg N ha}^{-1} \text{ yr}^{-1}$  (Ponette-González et al., 2021).

238 Outside of these specific ecoregions, the compilation of large datasets is beginning to allow for  
239 analysis of species and community responses to N across a region or their entire range (Thomas et  
240 al., 2010; Horn et al., 2018; Geiser et al., 2021). Herbaceous vegetation plots were compiled from  
241 studies that sampled species density and richness across a deposition gradient. The sites were  
242 differentiated between open and closed canopy ecosystems, and locations were skewed toward the  
243 eastern and Midwestern regions of the United States. Fewer plots were located in the Rocky  
244 Mountains, Southwest, and Great Plains regions. The relationship between N and species richness  
245 showed a unimodal relationship, suggesting that there was an initial increase in species numbers  
246 before richness declines. The critical load of N for a decline in species richness for open canopy  
247 systems was calculated based on local pH, precipitation, and average temperature and ranged from  
248  $7.4\text{--}10.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Simkin et al., 2016).

249 Clark et al. (2019) took the individual species locations from the open and closed canopy sites to  
250 evaluate their individual responses to N deposition. Of the 348 species analysed 198 were modelled  
251 to have either a decreasing, increasing, or unimodal response across the range of N deposition. 85%  
252 of the 198 species were negatively associated with N and/or S somewhere in the contiguous United  
253 States based on recent deposition model outputs. The average CL of N for the 198 species was  $11.2$   
254  $\text{kg N ha}^{-1} \text{ yr}^{-1}$  lining up with the critical load identified above for a decline in community richness  
255 begins to decline. Although, when species responses were evaluated across multiple vegetation  
256 alliances, over half had a variable response to nitrogen suggesting local factors play a role in  
257 sensitivity (Wilkins et al., In press). Species level responses are important for land managers as it



258 allows them to directly tie a response into a species of interest in their area. Clark et al. (2017)  
259 highlighted how changes in biodiversity and fire regimes can impact a slew of ecosystem services in  
260 grasslands across the country.

261 The dominant form of N deposition is changing from oxidized N to reduced N due to increased  
262 emissions for agricultural areas and mobile emission sources (Li et al., 2016; Nopmongcol et al.,  
263 2019). This shift will change the areas of highest deposition due to a shift in dominant sources of  
264 pollution as well as may impact the response of vegetation based on the deposition type (Stevens et  
265 al., 2011; van den Berg et al., 2016), further research is needed to understand what the impact of  
266 this will be. Continued advancement in the plasticity of species based on environmental conditions  
267 and community structure will help with the local application in management and policy in response  
268 to new and modified sources of pollution.

269

#### 270 **4. South America**

271 South American grasslands are highly diverse in terms of the vegetation structure, climate, altitude  
272 and edaphic characteristics (Bustamante et al., 2006; Blair et al., 2014; Dixon et al., 2014). Following  
273 Blair et al. (2014), we have used a broad grassland term to include ecosystems with a cover  
274 dominated by grasses and herbaceous vegetation, in some cases interspersed, with woody  
275 vegetation varying in the density of trees and/or shrubs. Although several South American  
276 grasslands have high conservation value because they exhibit high levels of biodiversity (Stevens et  
277 al., 2015; Dinerstein et al., 2017) and a high degree of endemism, they are endangered (Dinerstein et  
278 al., 2017). Increases in cover and abundance of woody species (Blair et al., 2014) and the conversion  
279 of grassland into agricultural or livestock land have reduced aboveground biomass (Kozovits and  
280 Bustamante, 2013) and N cycling (Reis et al., 2020). Overall, South American grasslands soils have  
281 low nutrient content and pH (López-Hernández, 2013; Borghetti et al., 2019). N deposition in South  
282 America is low in many regions but there are hotspots in Brazil (Ackerman et al., 2019). Fertilization  
283 experiments combining N and other nutrients have demonstrated that some fertilizers such as  
284  $(\text{NH}_4)_2\text{SO}_4$  caused a decrease in soil pH and an increase in Al levels (Lopes, 1998 in Bustamante et al.,  
285 2012). However, to what extent these results reflect the real impacts of the N atmospheric  
286 deposition on grasslands is not well understood.

287 Measurements of N emissions (Della Chiesa et al., 2019), N deposition, and its impacts on South  
288 American ecosystems are very scarce (Ometto et al., 2020). Additionally, most studies and  
289 observations are short-term (Ometto et al., 2020; Vet et al., 2014). Here we have used the

290 Dinerstein et al. (2017) ecoregions and biomes classification to focus on the impacts of N deposition  
291 on grassland cover without including those on trees/shrubs layer.

292 Grasslands from the Tropical and Subtropical Grasslands, Savannas, and Shrublands biome,  
293 specifically the Cerrado Domain in Brazil, have been more studied than those from other biomes.  
294 They are very heterogeneous and the forest-savanna transition is complex. It has been suggested  
295 that an increase in N deposition could promote woody encroachment and some savannas may  
296 transition to forest vegetation (Bueno et al., 2018). However, we did not find evidence to support  
297 this impact, instead the main effect was on vegetation biomass. Evidence of short and long-term  
298 (one year and 10 years-long) N fertilising experiment carried out in cerrado sensu stricto  
299 demonstrated that N-addition ( $100 \text{ kg N ha}^{-1}\text{yr}^{-1}$  as  $(\text{NH}_4)_2\text{SO}_4$ ) increased the cover and aboveground  
300 biomass of a native  $\text{C}_3$  grass, while the frequency of both native  $\text{C}_4$  grasses and the other monocots  
301 group (sedges, bromeliads and small palm trees) declined in the long-term and in the second  
302 sampling period, respectively (Bustamante et al., 2012). Another study also revealed changes in  
303 plant biomass. In a secondary savanna in Venezuela, which was a forest area more than 100 years  
304 ago and was then colonized by native grasses and sedges, results from a short-term (<1 year)  
305 fertilization experiment ( $200 \text{ kg ha}^{-1}\text{yr}^{-1}$  N) showed that N-addition resulted in an increase of the  
306 aboveground biomass of monocots and sedges. The latter group increased also from 4 to 8 percent  
307 of the community, these responses did not lead to a considerable change in species composition  
308 (Barger et al., 2002). In contrast there were few impacts of N addition on plant growth a short-term  
309 N deposition (one year) experiment which combined N addition ( $25 \text{ kg N ha}^{-1}\text{yr}^{-1}$ ) with water in  
310 cerrado ralo. Water addition was to mimic increased dry season precipitation in accordance with  
311 climate change scenarios predicted for the region. In one of the dominant native  $\text{C}_4$  grass species, N  
312 and water increased the probability of flowering and the number of flowering tillers in those  
313 individuals that did flower, while N-addition was negatively correlated with flowering for the other  
314 dominant species. These results, along with the foliar P recorded in the species studied, suggest that  
315 species-specific responses to the increases in N deposition and precipitation could impact ecosystem  
316 functioning via feedbacks between plant and soil P (Copeland et al., 2012).

317 Grasslands from the Flooded Grasslands and Savannas biome have been studied in the Venezuelan  
318 Llanos, results also revealed N deposition did not have an impact on plant growth. In a flooded  
319 savanna, Sarmiento et al. (2006) conducted a short-term (<1 year) fertilization experiment ( $50 \text{ kg}$   
320  $\text{ha}^{-1}$  N as urea). N-addition, at the start of the growing season, led to increase leaf N concentration.  
321 However, it did not increase plant growth and aboveground primary production. Overall, authors  
322 concluded that the vegetation is so well adapted to highly dystrophic soils that pulses of nutrient  
323 addition acts probably as a disturbance rather than as a factor releasing nutrient stress.

324 Research on grasslands from the Temperate Grasslands, Savannas, and Shrublands biome has been  
325 carried out in the Patagonian steppe (Argentina), an arid ecosystem dominated by C<sub>3</sub> species  
326 (Yahdjian et al., 2014; Flombaum et al., 2017). Results of a two-year N fertilization experiment (50 kg  
327 N ha<sup>-1</sup>yr<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>) showed an effect on plant biomass, grass foliar N concentration and grass  
328 biomass increased when N availability increased whereas shrubs did not respond (Yahdjian et al.,  
329 2014). Data from this study were subsequently analysed along with data from other global-change  
330 drivers, demonstrating that the effect of N deposition on aboveground net primary production was  
331 relatively low in comparison to the effect of biodiversity loss, increased grazing intensity and  
332 decreased precipitation (Flombaum et al., 2017).

333 Finally, we did not find any studies on the impacts of N deposition on grasslands from the Montane  
334 Grasslands and Shrublands, highlighting a knowledge gap for the South American region.

335 Although it is necessary to improve our understanding about the impacts of N deposition on all  
336 South American grassland types, there is a lack of information specifically for the Cerrado grassland  
337 formations (Campo Sujo, Campo Limpo and Campo Rupestre), The Pampa, Pantanal, The Argentine  
338 Monte, The Humid Chaco and the Montane grasslands and shrublands biome. The Nitrogen Human  
339 Environment Network within The Latin America Regional Centre of the International Nitrogen  
340 Initiative is carrying out research that will contribute to filling some of these gaps (Ometto et al.,  
341 2020). Impacts of N deposition on soils in this region are poorly understood. There is a lack of  
342 empirical data on effects of N-addition on biological N fixation rates prevents us estimating whether  
343 a decline of free-living biological N fixers will occur in grassland as it was recorded in tropical and  
344 subtropical moist forests in response to N-addition (Reis et al., 2020). The effects of N deposition on  
345 plant-microbe interactions have also not been investigated. To deal with some of these gaps it is  
346 necessary to initiate experiments and studies using the natural gradient of N deposition.  
347 Experiments need to apply low N-addition rates based on the current and projected deposition  
348 (Bobbink et al., 2010; Reis et al., 2020) and run over multiple years.

349 Another main gap is lack of knowledge about the critical N-loads for South American grasslands. In  
350 the short-term, to define the critical loads, we can use a steady-state models approach by combining  
351 data and knowledge of experts to determine thresholds for each type of grassland (Bobbink et al.,  
352 2010). In the mid and long-term, we can use the empirical critical N-loads approach to quantify how  
353 sensitive the grasslands are to N deposition (Bobbink et al., 2010). To use this approach, it is  
354 necessary to have evidence from the field about changes in ecosystem structure and function  
355 (Bobbink et al., 2010).

356

357 **5. Asia**

358 There are a variety of grasslands in Asia with different vegetation characteristics driven by the broad  
359 range of climatic and edaphic conditions throughout the continent, primarily distributed in Russia,  
360 Mongolia, China, Kazakhstan and middle Asia, Japan, and South Asia. Succession in natural and semi-  
361 natural grassland habitats is generally prevented by regional climate (e.g. temperature, solar  
362 insolation, and precipitation), soil conditions (e.g. pH, texture, and nutrient content), continuous or  
363 frequent natural disturbances (e.g. burning, floods, and grazing) as well as intensive human activities  
364 (e.g. mowing, grazing, and other management practices) (Goldstein and DellaSala, 2020). There are  
365 three main grassland types, based on the temperature, namely alpine (e.g. Tibet Plateau), temperate  
366 (e.g. the Steppes) and tropical (e.g. Terai-Duar, South Asian) grasslands. N deposition in Asia is the  
367 highest globally, with highest values seen in southern and western countries (Ackerman et al., 2019).  
368 Quite a few field surveys along N deposition gradients and N addition experiments have been  
369 conducted to understand the impacts of N deposition on natural/semi-natural grasslands in Asia,  
370 especially in China, over the past two decades (Soudzilovskaia and Onipchenko, 2005; Srinivasan et  
371 al., 2012; Verma et al., 2015; Han et al., 2019; Palpurina et al., 2019; Lin et al., 2021; Lu et al., 2021).

372 Temperature steppe constitutes the main part of grasslands in Asia, belonging to the Eurasian  
373 steppe and mainly hosted by Western Russia, Siberia, Kazakhstan, China, and Mongolia (Squires et  
374 al., 2018), and is also a research hotspot concerning on N deposition and biodiversity. Negative  
375 impacts of N deposition on species richness were widely observed in temperate steppes (Fang et al.,  
376 2012; Song et al., 2012), as well as increased aboveground biomass (Bai et al., 2010; Zhang et al.,  
377 2015) and decreased belowground biomass (Bai et al., 2015; Xu et al., 2017) with N addition. Bai et  
378 al. (2010) found that species richness decreased with N addition in temperate steppes, especially  
379 for annuals and perennial rhizome grasses; there was a lower critical threshold for N-induced species  
380 loss in a mature steppe ( $17.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) than in a degraded steppe ( $52.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ). Species  
381 loss with N addition was modified by precipitation and sampling areas, being greater in wet years  
382 than dry years (Lan and Bai, 2012) but less at larger scale (Lan et al., 2015), suggesting climate/scale-  
383 dependent N impacts on biodiversity. Hao et al. (2018) reflected the cumulative and recoverable  
384 negative impacts of N addition on species richness in a semiarid temperate steppe, that is low N  
385 deposition/inputs could cause a significant species loss with N accumulation and further recycle over  
386 time. After the cessation of N addition ( $480 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), species richness rapidly recovered to and  
387 maintained 50-70% of that in the control plots with decreased concentrations of soil inorganic N and  
388 foliar N. Limited recovery from N addition might be due to soil acidification (soil pH decreased by  
389  $\sim 1.5$  units) and the absence of mowing. Further research found that mowing (removal of plant-N)  
390 could maintain diversity from N-induced species loss in the temperate steppe (Yang et al., 2019. ; Lu

391 et al., 2021). Eutrophication and acidification are two primary mechanisms driving plant species loss  
392 under enhanced N deposition. However, Tian et al. (2016) showed a soil manganese (Mn) related  
393 mechanism of N-induced species loss in a temperate steppe, where foliar Mn concentrations in forbs  
394 were 10 times higher than that in grasses following N-induced soil acidification and enhanced  
395 available Mn concentrations in soil, limiting the photosynthetic capacity of forbs.

396 Alpine grasslands in Asia, characterized by high solar radiation and low temperature with a large  
397 diurnal range, have received increasing attention to examine the N-impacts on species loss in the  
398 past decade, especially on Tibetan Plateau (Fu and Shen, 2016; Li et al., 2019; Wang et al., 2020).  
399 Yang et al. (2015) provided field evidence to confirm abundance- and functional-based mechanisms  
400 of N-induced species loss in an alpine meadow, while abundance-based mechanisms became more  
401 dominant if herbivores existed. However, plant species richness, as well as aboveground biomass,  
402 was observed to be not sensitive to N addition in an alpine grassland in the mid-Tianshan mountains  
403 of Central Asia (Li et al., 2015), which could be true if there are other resources limitations (e.g.  
404 water, temperature, or nutrients) or weak N-induced competition exclusions (light in especial) due  
405 to low species richness and density pressure. Similar weak species responses to N addition were also  
406 observed in the five-year N addition experiments in four alpine grasslands (alpine meadow, alpine  
407 meadow steppe, alpine steppe, and alpine desert-steppe, with a general low species richness (<10  
408 species per m<sup>2</sup> in the control plots) along precipitation gradients on Tibetan Plateau (Zong et al.,  
409 2019). In contrast, significant species loss with N addition was observed in a Tibetan alpine meadow  
410 with higher species richness (~20 species per m<sup>2</sup>) (Niu et al., 2014), reflecting competitive exclusion  
411 as a mechanism of species loss.

412 Tropical and subtropical grasslands (like Terai-Duar) in Asia are characterized by high annual  
413 precipitation, hot and humid climate especially during the summer monsoon. Few studies have been  
414 carried out to determine the impacts of N deposition on species diversity in tropical and subtropical  
415 grasslands (Sagar et al., 2015; Verma et al., 2015). Verma and Sagar (2020) found a humped-shape  
416 relationship between species richness and N addition rates in a tropical grassland, and significant  
417 species loss only occurred at the N addition rate of 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The number of annual and non  
418 N-fixing species increased with N addition, as well as short stature species until N addition rate  
419 increased to 120 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Verma et al., 2015), suggesting a high critical threshold of N-induced  
420 species loss in tropical grasslands in spite of competition mechanism.

421 Grasslands in Japan are distributed throughout subarctic-subtropic zones. Although N deposition in  
422 Japan is around the N critical loads for species loss, few studies have revealed the impacts of N  
423 deposition on species diversity (Lin et al., 2021), requiring further research.

424 Through many studies on a large spatial and temporal scale, there is a good understanding of species  
425 diversity in response to N deposition in grasslands in parts of Asia. With raised awareness of N  
426 pollution, implemented N emission controls and requirement for global carbon neutrality, N  
427 deposition will likely decrease in the near future, exactly as what Europe has experienced. However,  
428 there is a lack of understanding regarding ecological recovery of grasslands from N deposition in  
429 Asia. Many of the cited experiments use high levels of N addition. Significant soil acidification was  
430 observed in major northern China's grasslands (Yang et al., 2012), this is an important mechanism of  
431 N-induced species loss and thus liming effects on species diversity are interesting to know in severe  
432 acidified grasslands. In addition, experimental N addition with care should be also considered to  
433 reduce the amplified N impacts in manipulative experiments, caused by N addition at high rates and  
434 low frequency (Zhang et al., 2014), to better predict species diversity in the future scenarios of N  
435 deposition.

## 436 **6. Africa**

437 African savannas and grasslands are ancient ecosystems (Bond, 2016; Bond et al., 2019; Nerlekar  
438 and Veldman, 2020) that are high in diversity and well adapted to nutrient-poor soils (Silveira et al.,  
439 2020). They are characterised by heterogeneity of climate, nutrient resources and disturbance  
440 (Sankaran et al., 2005) on ancient, often highly weathered soils that don't necessarily reflect the  
441 characteristics of the parent material (Menault et al., 1985). Soil nutrient levels are extremely  
442 variable across the savanna and grassland regions of Africa, with rainfall inversely linked to soil pH  
443 and nutrient availability (Hengl et al., 2017). African savannas and grasslands are largely exposed to  
444 frequent fires and are consequently adapted to regular fires and the impacts of those fires. One of  
445 those impacts is the potential reduction of soil nutrients, particularly N (Fynn et al., 2003; Gray and  
446 Bond, 2015), further enhancing the vegetation adaptation to low soil N levels.

447 Nitrogen deposition rates and extent in Africa are less well quantified than for other continents,  
448 particularly those in the northern hemisphere. Ackerman et al. (2019) show relatively low rates of N  
449 deposition across most of Africa, but do show relatively high rates of change in deposition levels,  
450 indicating an increasing magnitude of deposition. Other studies show varying rates of N deposition in  
451 different regions, as influenced by proximity to industrialised zones or vegetation burning (Bauters  
452 et al., 2018; Mompoti, 2019; Ossouhou et al., 2021). It is likely that, with increasing levels of  
453 industrialisation in Africa, N deposition rates will increase.

454 Various long- and short-term studies have been carried out to determine the impacts of nutrient  
455 additions (including N) on grasslands and savanna composition, structure and productivity. These  
456 studies are mainly centred in southern and eastern Africa. While the levels of added N in these

457 studies may not necessarily approximate deposition levels, they nonetheless indicate trends of  
458 response to N addition, thus allowing for developing realistic models for assessing impacts of  
459 deposition.

460 In a comprehensive, on-going, long-term nutrient addition experiment (Ukulinga Grassland Nutrient  
461 Experiment) initiated in 1950 in a mesic South African grassland, above-ground net primary  
462 productivity (ANPP) averaged 337 (no added nutrients), 428 (N added) and 518 (N and P added) g.m<sup>-2</sup>  
463 over a 30 year evaluation period (Fynn and O'Connor, 2005). ANPP was responsive to N addition  
464 and the interaction between N and P. Nitrogen addition also reduced soil pH, which contributed to a  
465 reduction in forb richness and density (Fynn and O'Connor, 2005; Tsvuura and Kirkman, 2013). The  
466 soil pH dynamics were further examined by varying sources of N and liming treatments incorporated  
467 in the experimental design (le Roux and Mentis, 1986). Soil mycelial biomass increased with N  
468 addition, but was unresponsive to other nutrients (Tsvuura et al., 2017). Soil respiration was found  
469 to decrease with increasing soil N levels and also decreased with declining soil pH on the same  
470 experiment (Ward et al., 2017a). This response was evident in the long-term (60+ years of treatment  
471 application) Ukulinga Grassland Nutrient Experiment, but not in the adjacent Nutrient Network  
472 (NUTNET) experiment (~10 years of treatment application). The grass species in question have been  
473 shown to exhibit trade-offs in competitive ability along soil fertility gradients (Fynn et al., 2005) and  
474 in this experiment plots without added nutrients were dominated by short, narrow leaved grass  
475 species, plots with added N were dominated by tall, narrow-leaved species and those with added N  
476 and P were dominated by tall, broad-leaved species. Complementary research confirmed that grass  
477 specific leaf area, height and shade tolerance were related to competitive success at higher soil  
478 nutrient levels (Fynn et al., 2011). Strong indications of co-limitation by P on ANPP were noted in this  
479 experiment (le Roux and Mentis, 1986; Fynn and O'Connor, 2005; Tsvuura and Kirkman, 2013).

480 These generalised responses have been confirmed in other studies in South Africa and further afield  
481 in Africa. Snyman and Oosthuizen (2001) recorded rapid changes in species composition from a  
482 climax state to a sub-climax state with three years of N application, with a concomitant increase in  
483 ANPP in a semi-arid grassland (MAP 560 mm). The authors also noted the role of P as a co-limiting  
484 nutrient. Craine et al. (2008) assessed the impacts of added N and P on ANPP across a rainfall  
485 gradient (MAP 457 – 737 mm) in the Kruger National Park in South Africa. They found a similar  
486 pattern across all five of their sites, with N addition increasing ANPP to a greater extent than P  
487 addition, but the combination of N and P showed the greatest increase in ANPP. Responses were  
488 unrelated to N and P levels and ratios in the vegetation. In an east African savanna (MAP 650 mm),  
489 ANPP responded in a similar manner to N and P addition in open savanna away from trees. Under  
490 leguminous tree canopies, where N fixation raised soil N levels, P emerged as the primary limiting

491 nutrient (Fulco et al., 2001). An experiment investigating the interaction of defoliation and N  
492 addition on a short grass from the Serengeti in east Africa showed a similar response of N addition  
493 boosting ANPP (William Hamilton III et al., 1998).

494 While the majority of the information presented for African savannas and grasslands emanates from  
495 South Africa, the limited data from elsewhere in Africa indicates convergence of responses to N.  
496 Several comparisons with sites on other continents likewise show inter-continental convergence of  
497 responses. In comparison between the Ukulinga Grassland Nutrient Experiment (1950 - ) and the  
498 Park Grass study at Rothamstead in the UK (1856 - ), showed a significant additive interaction of the  
499 effects of N and P addition on ANPP. Nitrogen amount was negatively related to species richness, in  
500 contrast to the positive relationship with soil pH (Ward et al., 2017b; Ward et al., 2020). In a direct  
501 comparison of the impacts of N addition on species richness on mesic grassland subjected to varying  
502 long-term fire treatments between the Ukulinga Grassland Fire Experiment (initiated in 1950) and  
503 the Konza Prairie Biological Station (fire experiment initiated in 1977), N was added at a rate of 100  
504 kg ha<sup>-1</sup> for four years on both sites on unburned, intermediate fire frequency and annually burned  
505 treatments. While both sites showed a decline in species richness in response to the add N, the  
506 magnitude of species loss was greater on the South African site (Kirkman et al., 2014). In a similar  
507 study, which included sites in the Kruger National Park in South Africa, Buis et al. (2009) quantified  
508 the reduction in soil N in frequently burned sites, which was closely related to the consequent  
509 response of ANPP to added N in the frequently burnt treatments, with a relative lack of response in  
510 the unburnt treatments with higher inherent soil N levels. The comparisons between South African  
511 and North American savannas and grasslands indicate that N addition changes species composition,  
512 reduces species richness and increases ANPP (Smith et al., 2016).

513 Examining the responses of African sites in the international Nutrient Network (NUTNET) confirmed  
514 that N is the key nutrient limiting ANPP, with P the main co-limiting nutrient (Fay et al., 2015) while  
515 also reducing species richness (Harpole et al., 2016). This points to generalised responses of ANPP  
516 and species richness to added N, taking the influence of climate into account (Flores-Moreno et al.,  
517 2016).

518 Plant traits, including plant height and specific leaf area likely influence competitive interactions in  
519 the presence of added N to increase ANPP and eliminate species unable to compete effectively  
520 (Fynn et al., 2005; Pennings et al., 2005; Fynn et al., 2011).

521 Lack of spatial representation of experiments across African grasslands and savannas inhibits  
522 comprehensive coverage of responses to added N. However, the convergence of impacts within



523 experiments in Africa and in comparative experiments with sites on other continents indicates a  
524 general convergence of responses.

525

## 526 **7. Australasia**

527 Grasslands occupy a large portion, possibly greater than 50%, of the coastal and interior areas of the  
528 Australian continent (Mclvor, 2005; Bell et al., 2014). Due to the high diversity of climatic conditions  
529 found across Australia, the variety and biodiversity of their grasslands is immense, from the desert  
530 and Mediterranean grasslands of South and Western Australia dominated by tussock and annual  
531 grasses to the fertile mesic grasslands of the lowlands such as those in New South Wales and  
532 Victoria, the alpine grasslands of the Snowy Mountains, and the tropical savanna and wet grasslands  
533 of Queensland and the Northern Territory (Mclvor, 2005; Dixon et al., 2014). The varied geology and  
534 the characteristic old age of many Australian soils, which typically results in very nutrient-deficient  
535 soils (Mclvor, 2005; Lambers et al., 2011), including low contents of N, P, and various trace metals.  
536 Together with the isolated evolutionary history of their native plant communities (Broadhurst and  
537 Coates, 2017) this low nutrient status contributes to the high species richness and degree of  
538 endemism of Australian grasslands. Until the arrival of the first Europeans, Australian grasslands  
539 were maintained by the presence of macropods, including kangaroos and wallabies, as well as by the  
540 management of Australian aboriginals, who used fire to control the encroachment of woody  
541 vegetation (Noble et al., 2007). Currently, grasslands in Australia are maintained by communities of  
542 native, feral (e.g., rabbits, dromedaries, donkeys, and horses), and domesticated (e.g., sheep and  
543 cattle) animals (Noble et al., 2007), while many former grasslands have now been transformed into  
544 pasture, and many of these are overgrazed (Bell et al., 2014; Broadhurst and Coates, 2017).

545 N deposition is not considered as a widespread threat to the biodiversity and integrity of Australian  
546 ecosystems, including grasslands and deposition is typically very low. The potential ecological  
547 consequences of climate change (Lindenmayer et al., 2010), land use change (Bell et al., 2014;  
548 Broadhurst and Coates, 2017), including urbanization and overgrazing, and species invasion of both  
549 plant and animal communities (Morgan, 1998; Mclvor, 2005) of much greater concern. This lack of  
550 concern is reflected in the scarcity, almost completely absence, of scientific literature studying the  
551 potential impacts of N deposition on Australian grasslands, either in the form of experimental N  
552 addition studies simulating realistic doses, or in the form of observational studies across real  
553 gradients. For example, a recent meta-analysis of 174 publications evaluating N deposition in urban  
554 areas did not include any single study from Australia and the Pacific Islands (Decina et al., 2020). This

555 is due to the fact that most of the country is devoid of human population, and population  
556 concentrations are very low across most of the country, which results in limited emissions of N  
557 compounds that are usually associated with large cities (Ayers et al., 1995; Dentener et al., 2006).  
558 Moreover, most of the big Australian cities are located upwind of polluting sources, resulting in an  
559 export of pollutants to the Pacific Ocean and its islands (Stohl et al., 2002; Longley et al., 2019). The  
560 general lack of water also limits the implementation of intensive agriculture across much of the  
561 territory, thus resulting in somewhat limited emissions of reduced N across the region. The most  
562 likely impacts of N deposition on Australian grasslands are thus expected to be linked to point  
563 sources (Ayers et al., 1995; Shen et al., 2016), including poultry, swine, and cattle farms, intensive  
564 agricultural operations. This is particularly the case across the most populated regions and the wheat  
565 belt, and also dust particles transported by the wind across long distances originating in China,  
566 Southeast Asia, and to a lesser extent, Africa (Stohl et al., 2002). Open-air mines are also another  
567 potential source of N to the atmosphere (Hendryx et al., 2020), but in this case the ecological  
568 impacts of N would be also associated with those of other polluting elements such as toxic metals.  
569 Anyhow, estimates of N deposition across Australian grasslands are very rare, and thus our best  
570 estimates are currently based on global models; current estimates are around  $1\text{-}5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$   
571 (Dentener et al., 2006). Consistent with the low importance of N deposition in Australia, the rate of  
572 N creation across the last 60 years in Oceania is the lowest as compared to the rest of continents  
573 (Galloway et al., 2021).

574 Some studies evaluating the impacts of increased N on Australian grasslands exist, but they do not  
575 add N at realistic doses. Many of these studies have been carried out in the context of the Nutrient  
576 Network (NutNet), which is a coordinated network of experimental sites following the same  
577 fertilization protocol (Borer et al. 2017). In this case, N was added at a rate of  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ,  
578 alone or in combination with other nutrients such as P, K, and other macronutrients and trace  
579 elements (Borer et al. 2017). Despite these high doses, Ochoa-Hueso et al. (2019) did not find effects  
580 of one year of N addition on litter decomposition, soil activity, and soil bacterial and fungal  
581 abundance in a mesic grassland that was previously used for cattle grazing near the Blue Mountains,  
582 New South Wales. Another study carried out across four Australian grasslands showed that 6 years  
583 of fertilization with N, plus P, K and other essential macro and micronutrients, did not affect  
584 grassland productivity, exotic cover, or exotic richness change across time, but reduced native  
585 richness, suggesting potential negative effects of ecosystem eutrophication (Standish et al., 2012).  
586 However, responses from Standish et al. (2012) were site-dependent, with the low rainfall  
587 Mediterranean site where annuals were dominant being the only site displaying year-dependent  
588 increases in biomass in response to fertilization. Exotic species were the most important plant group

589 contributing to this response. For other parts of Australasia we were not able to identify any studies  
590 on impacts of N deposition on grasslands.

591 Beyond the lack of information regarding the potential impacts of increased N deposition at realistic  
592 doses, the very special case of Australian grasslands represents an invaluable opportunity to study  
593 the sensitivity of native ecosystems that have not been so far extensively exposed to this threat,  
594 something that is now virtually impossible in other continents like Europe, America or Asia. For  
595 example, in Europe the most dramatic impacts of N deposition on grasslands are thought to have  
596 occurred decades or even more than a century ago, resulting in currently highly altered, and  
597 impoverished communities (Bobbink et al., 2010). Thus, one potential goal of futures studies aimed  
598 at manipulating N in realistic doses across Australian grasslands could be understanding the way in  
599 which grassland communities shift during the first transitioning phases, and the speed at which the  
600 most sensitive plant species are lost at such low doses. Another relevant aspect to consider is the  
601 potential importance of environmental factors such as water and nutrient availability other than N  
602 as modulators of the response of Australian grasslands to N deposition. We hypothesize that the  
603 typically extremely low availability of other macronutrients such as P, K, and trace elements may  
604 play a particularly important role in this response, potentially by limiting the response of plants, and  
605 their associated microbes, to the extra N, which they may not be able to use effectively. Similarly,  
606 the typically coarse texture of Australian grassland soils, and the low amount and unpredictability of  
607 rainfall events, may also mean a reduced ability of Australian grasslands to respond to increased N  
608 due to low retention capacity. However, such climatic and soil characteristics may, in turn, mean a  
609 greater leaching potential and thus a greater sensitivity of phreatic waters to increased N deposition.

610

## 611 **8. Conclusions**

612 It is clear from this review and other research that N deposition impacts on grasslands can be  
613 observed to greater or lesser extents across the world. Research effort into the impacts of nitrogen  
614 deposition on grasslands is not evenly distributed across the globe. We have a good understanding  
615 of impacts in some parts of the world, particularly Europe, North America and parts of Asia but much  
616 less understanding in other regions. In some areas, where deposition is low and likely to remain so  
617 into the future, this reflects the lack of pressure from N deposition as a driver of vegetation change  
618 but in other areas it reflects a lack of resources. Despite variation in our knowledge and  
619 understanding there are clear themes that emerge including around increased biomass production  
620 and reduction in plant species richness as N deposition increases. These common responses mean

621 that we can predict responses of grasslands in areas where we have little evidence of impacts.  
622 However, a research priority is identifying areas where N deposition is above critical loads used in  
623 other regions but impacts are not understood and identifying areas where N deposition is likely to  
624 increase and ensuring monitoring is in place (Table 1). In some regions, including parts of Europe and  
625 North America we are beginning to see declines in N deposition making understanding prospects for  
626 recovery an important area of research. Understanding interactions between N deposition and  
627 climate change is another important area where we currently have a number of knowledge gaps  
628 (Borer and Stevens, 2022).

629

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1127

1128 **Table 1. Summary of key knowledge gaps and research priorities in each region.**

| <b>Continent</b> | <b>Research priorities and knowledge gaps</b>   |
|------------------|---|
| Europe           | <ul style="list-style-type: none"> <li>• Changes in the ratio of reduced and oxidised N inputs</li> <li>• Recovery from N deposition</li> </ul>   |
| North America    | <ul style="list-style-type: none"> <li>• Changes in the ratio of reduced and oxidised N inputs</li> <li>• Individual species responses</li> </ul>   |
| South America    | <ul style="list-style-type: none"> <li>• Improved understanding of impacts of N on major grassland types</li> <li>• Understanding the role of N fixers and other plant soil interactions</li> <li>• Critical loads for major grassland types</li> </ul> |
| Asia             | <ul style="list-style-type: none"> <li>• Recovery from N deposition</li> <li>• Experiments with low N inputs and high frequency additions to refine predictions of community response</li> </ul>  |
| Africa           | <ul style="list-style-type: none"> <li>• Improved understanding of impacts of N on major grassland types</li> </ul>   |
| Australasia      | <ul style="list-style-type: none"> <li>• Improved understanding of impacts of N on major grassland types</li> <li>• Interactions between N deposition and water availability and other nutrients</li> </ul>   |

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1131 **Figure legends**

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1133 **Figure 1.** The relative area of grasslands within each continent (Loveland et al., 2000) under  
1134 increasing levels of total N deposition as defined by Ackerman et al. (2019).

1135 **Figure 2.** Mechanisms by which atmospheric nitrogen deposition has negative impacts on grassland  
1136 vegetation. Many of the mechanisms operate over long-timescales or a combination of short-and  
1137 long-timescales although direct toxicity would tend to occur over shorter timescales.

1138

1139 **Graphical abstract**

1140 Global grasslands: Clockwise from top right Nutnet N addition experiment in Richmond, Australia; N  
1141 addition experiment in Duolun, China; Calcareous grasslands and pastures in Arncliffe, UK; Grassland  
1142 on the Inca Train, Peru; Centre: Great Plains, USA.