Research progress on the impact of nitrogen deposition on global grasslands

Running title: Nitrogen deposition and grasslands

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Highlights

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

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

Keywords

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

1. Introduction

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

Globally, nitrogen (N) deposition exceeds 90 Tg N yr\(^{-1}\) (Ackerman et al., 2019; Figure 1) which has led to a wide range of impacts in grasslands. There are two main methods used to investigate the impacts of N deposition on grasslands. The first is experimental additions of N. This approach commonly uses a replicated plot design and offers the advantage of being a controlled experiment where confounding factors are minimised but the disadvantage that it is hard to replicate N addition as it would occur in the environment. However, many experiments use high levels of N addition to mimic longer-term responses on a short timescale. Experiments can be hard to maintain long-term
but fortunately there are many examples of long-term N addition experiments across the world (e.g. Clark et al., 2009; Basto et al., 2015; Hou et al., 2020). A second, increasingly common approach is to utilise gradients of N deposition within the environment, either around point sources of N emission (e.g. Pitcairn et al., 1998) or across large landscape or regional gradients (e.g. Stevens et al., 2010a). Using a gradient of N deposition can present challenges in terms of disentangling variables likely to impact on soils and vegetation but offers the advantage of demonstrating real-world impacts.

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

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

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

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

Grasslands with a neutral pH present a similar picture to acidic grasslands where research has demonstrated declines in species richness associated with increasing N deposition with fewer forbs and species considered indicators of low nutrient status (Roth et al., 2017; Boch et al., 2021). The world’s longest running experiment investigates the impacts of nutrients to a mesic grassland. The Park Grass experiment was initiated in 1856 by John Lawes and Joseph Gilbert in a neutral hay meadow to investigate how different fertilisers impacted yield in hay meadows (Silvertown et al., 2006). The longevity of this experiment means that it has become very useful for understanding the impacts of nutrients on a wide range of response variables. This experiment has been used to
understand both the impacts of N deposition on grasslands in terms of species composition and soils
(Goulding et al., 1998), and to assess recovery as N deposition is reduced (Storkey et al., 2015).

Whilst evidence for changes in species richness is less clear in calcareous grasslands (Maskell et al.,
2010; Tipping et al., 2013) there is clear evidence for a change in species composition with an
increase in grass cover and a decline in forbs (van den Berg et al., 2016). A number of studies
identified a decline in specialist and rare species (Van den Berg et al., 2011; Diekmann et al., 2014).

In Dutch calcareous grasslands considerable changes in species composition at high N were
associated with an increase in the abundance of the grass Brachypodium pinnatum (Bobbink and
Willems, 1987). Changes in the below-ground community have also been observed (Ceulemans et
al., 2019). In calcareous grasslands the well buffered soils are less likely to be susceptible to
acidification. Some calcareous grasslands show signs of phosphorus (P) limitation which reduced the
impact of N addition (e.g. Bonanomi et al., 2009) but this is clearly not always the case.

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

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

Whilst there are grassland habitats where further information is needed, in particular to support the
decision making process around critical loads (Bobbink and Hettelingh, 2011), within Europe the
bigger knowledge gaps exist around changes in the N deposited. Within Europe tighter legislative
control and co-benefits from carbon cutting measures have reduced N oxide emissions (Fowler et al.,
2004). While ammonia emissions have remained high in many regions this has led to a change in the
balance between reduced and oxidised ammonia with impacts on ecosystems likely (Stevens et al.,
2011). To date relatively few experiments have investigated the differential effects of reduced and
oxidised N. Furthermore, total deposition is beginning to be reduced in some areas, whilst there is a
lot of variability across Europe in the extent of reductions in deposition (Sutton et al., 2011) there is
considerable interest in how habitats will recover from N deposition. Whilst some studies have been  
conducted (Stevens, 2016) there is still a need for further evidence in this area.

3. North America

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

The northernmost grasslands on the continent are the tundra and the taiga. The Tundra ecoregion is  
on the northern edge of the continent and contains vast grassland-like plains among mesa and  
mountains. The low critical load range between 1–3 kg N ha\(^{-1}\) yr\(^{-1}\) aims primarily to protect against  
changes in shrub and grass cover (Pardo et al., 2011). The Taiga consists of broadly rolling uplands  
and lowlands with a mix of open grasslands, peatlands, and stunted forests and has a critical load of  
6 kg N ha\(^{-1}\) yr\(^{-1}\). Here the main effects of N that have been observed are changes in CO\(_2\) exchange,  
increase in foliar N, and change community composition of vascular plants (Pardo et al., 2011).

The Northwestern Forested Mountains ecoregion has grasslands in alpine and subalpine areas. N  
fertilization experiments within the sites in the alpine tundra of the Rocky Mountains showed an  
increasing cover of an alpine sedge species (Carex rupestris) with N additions greater than 3 kg N ha\(^{-1}\)  
yr\(^{-1}\) as well as an increase in soil NO\(_3\) leaching once fertilization exceeded 10 kg N ha\(^{-1}\) yr\(^{-1}\) (Bowman  
et al., 2012). Cessation of fertilization led to a decrease in the enhanced C. rupestris cover, but little  
recovery of soil processes over a decade of monitoring (Bowman et al., 2018). Pardo et al (2011) give  
a critical load for alpine grasslands between 4–10 kg N ha\(^{-1}\) yr\(^{-1}\), however, another study in Rocky  
Mountain National Park estimated a similar but more sensitive critical load of N for protecting  
subalpine vegetation from changes to biodiversity, between 1.9–3.5 kg N ha\(^{-1}\) yr\(^{-1}\) (McDonnell et al.,  
2014). This modelling study suggested that subalpine plant species cover in Rocky Mountain  
National Park has already changed by more than 10% due to N deposition over the past century and  
future climate change is expected to increase these changes.
The warmer, arid areas encompass the deserts and Mediterranean ecoregions. The North American Deserts ecoregion make up most of the western United States (USA) and northern Mexico, with variation of dominant shrubs and grasses within different desert boundaries. Critical loads range from 3–8.4 kg N ha\(^{-1}\) yr\(^{-1}\) for increased biomass of invasive grasses and a decrease of native forbs (Bobbink et al., 2010). Responses across desert environments have been inconsistent in these responses and often correlated with years of high precipitation (Kazanski et al., 2021). An N fertilization study on the Colorado Plateau did not affect plant diversity or the abundance of a common invasive grass, *Bromus tectorum*, in fertilization plots, but N did have a negative effect on biological soil crusts which may eventually confer changes to vegetation communities (Phillips et al., 2021). Yet in other studies in these cooler deserts, *B. tectorum* often does increase with elevated N depending on local soil properties (Belnap et al., 2016). Within the Sonoran/Chihuahua deserts of the southern USA and northern Mexico, there have also been mixed results on the impact of N deposition on grassland communities and the spread of one of the main species of concern, buffelgrass (*Cenchrus ciliaris*). Some grass dominated communities have shown resilience to vegetation changes with N additions in the absence of disturbance (Ladwig et al., 2012), but show an increase in forb growth and diversity after fire removed grass cover and water and N additions were continued (Collins et al., 2017). Another study has shown a neutral effect on *C. ciliaris* with negative effects on native vegetation, which can still lead to the accelerated spread of the invasive grass (Lyons et al., 2013).

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

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

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

Clark et al. (2019) took the individual species locations from the open and closed canopy sites to evaluate their individual responses to N deposition. Of the 348 species analysed 198 were modelled to have either a decreasing, increasing, or unimodal response across the range of N deposition. 85% of the 198 species were negatively associated with N and/or S somewhere in the contiguous United States based on recent deposition model outputs. The average CL of N for the 198 species was 11.2 kg N ha\(^{-1}\) yr\(^{-1}\) lining up with the critical load identified above for a decline in community richness begins to decline. Although, when species responses were evaluated across multiple vegetation alliances, over half had a variable response to nitrogen suggesting local factors play a role in sensitivity (Wilkins et al., In press). Species level responses are important for land managers as it
allows them to directly tie a response into a species of interest in their area. Clark et al. (2017) highlighted how changes in biodiversity and fire regimes can impact a slew of ecosystem services in grasslands across the country.

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

4. South America

South American grasslands are highly diverse in terms of the vegetation structure, climate, altitude and edaphic characteristics (Bustamante et al., 2006; Blair et al., 2014; Dixon et al., 2014). Following Blair et al. (2014), we have used a broad grassland term to include ecosystems with a cover dominated by grasses and herbaceous vegetation, in some cases interspersed, with woody vegetation varying in the density of trees and/or shrubs. Although several South American grasslands have high conservation value because they exhibit high levels of biodiversity (Stevens et al., 2015; Dinerstein et al., 2017) and a high degree of endemism, they are endangered (Dinerstein et al., 2017). Increases in cover and abundance of woody species (Blair et al., 2014) and the conversion of grassland into agricultural or livestock land have reduced aboveground biomass (Kozovits and Bustamante, 2013) and N cycling (Reis et al., 2020). Overall, South American grasslands soils have low nutrient content and pH (López-Hernández, 2013; Borghetti et al., 2019). N deposition in South America is low in many regions but there are hotspots in Brazil (Ackerman et al., 2019). Fertilization experiments combining N and other nutrients have demonstrated that some fertilizers such as (NH₄)₂SO₄ caused a decrease in soil pH and an increase in Al levels (Lopes, 1998 in Bustamante et al., 2012). However, to what extent these results reflect the real impacts of the N atmospheric deposition on grasslands is not well understood.

Measurements of N emissions (Della Chiesa et al., 2019), N deposition, and its impacts on South American ecosystems are very scarce (Ometto et al., 2020). Additionally, most studies and observations are short-term (Ometto et al., 2020; Vet et al., 2014). Here we have used the
Dinerstein et al. (2017) ecoregions and biomes classification to focus on the impacts of N deposition on grassland cover without including those on trees/shrubs layer.

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

Grasslands from the Flooded Grasslands and Savannas biome have been studied in the Venezuelan Llanos, results also revealed N deposition did not have an impact on plant growth. In a flooded savanna, Sarmiento et al. (2006) conducted a short-term (<1 year) fertilization experiment (50 kg ha\(^{-1}\) N as urea). N-addition, at the start of the growing season, led to increase leaf N concentration. However, it did not increase plant growth and aboveground primary production. Overall, authors concluded that the vegetation is so well adapted to highly dystrophic soils that pulses of nutrient addition acts probably as a disturbance rather than as a factor releasing nutrient stress.
Research on grasslands from the Temperate Grasslands, Savannas, and Shrublands biome has been carried out in the Patagonian steppe (Argentina), an arid ecosystem dominated by C3 species (Yahdjian et al., 2014; Flombaum et al., 2017). Results of a two-year N fertilization experiment (50 kg N ha\(^{-1}\) yr\(^{-1}\) as NH\(_4\)NO\(_3\)) showed an effect on plant biomass, grass foliar N concentration and grass biomass increased when N availability increased whereas shrubs did not respond (Yahdjian et al., 2014). Data from this study were subsequently analysed along with data from other global-change drivers, demonstrating that the effect of N deposition on aboveground net primary production was relatively low in comparison to the effect of biodiversity loss, increased grazing intensity and decreased precipitation (Flombaum et al., 2017).

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

Another main gap is lack of knowledge about the critical N-loads for South American grasslands. In the short-term, to define the critical loads, we can use a steady-state models approach by combining data and knowledge of experts to determine thresholds for each type of grassland (Bobbink et al., 2010). In the mid and long-term, we can use the empirical critical N-loads approach to quantify how sensitive the grasslands are to N deposition (Bobbink et al., 2010). To use this approach, it is necessary to have evidence from the field about changes in ecosystem structure and function (Bobbink et al., 2010).
There are a variety of grasslands in Asia with different vegetation characteristics driven by the broad range of climatic and edaphic conditions throughout the continent, primarily distributed in Russia, Mongolia, China, Kazakhstan and middle Asia, Japan, and South Asia. Succession in natural and semi-natural grassland habitats is generally prevented by regional climate (e.g. temperature, solar insolation, and precipitation), soil conditions (e.g. pH, texture, and nutrient content), continuous or frequent natural disturbances (e.g. burning, floods, and grazing) as well as intensive human activities (e.g. mowing, grazing, and other management practices) (Goldstein and DellaSala, 2020). There are three main grassland types, based on the temperature, namely alpine (e.g. Tibet Plateau), temperate (e.g. the Steppes) and tropical (e.g. Terai-Duar, South Asian) grasslands. N deposition in Asia is the highest globally, with highest values seen in southern and western countries (Ackerman et al., 2019). Quite a few field surveys along N deposition gradients and N addition experiments have been conducted to understand the impacts of N deposition on natural/semi-natural grasslands in Asia, especially in China, over the past two decades (Soudzilovskaia and Onipchenko, 2005; Srinivasan et al., 2012; Verma et al., 2015; Han et al., 2019; Palpurina et al., 2019; Lin et al., 2021; Lu et al., 2021). Temperature steppe constitutes the main part of grasslands in Asia, belonging to the Eurasian steppe and mainly hosted by Western Russia, Siberia, Kazakhstan, China, and Mongolia (Squires et al., 2018), and is also a research hotspot concerning on N deposition and biodiversity. Negative impacts of N deposition on species richness were widely observed in temperate steppes (Fang et al., 2012; Song et al., 2012), as well as increased aboveground biomass (Bai et al., 2010; Zhang et al., 2015) and decreased belowground biomass (Bai et al., 2015; Xu et al., 2017) with N addition. Bai et al. (2010) found that species richness decreased with N addition in temperature steppes, especially for annuals and perennial rhizome grasses; there was a lower critical threshold for N-induced species loss in a mature steppe (17.5 kg N ha\(^{-1}\) yr\(^{-1}\)) than in a degraded steppe (52.5 kg N ha\(^{-1}\) yr\(^{-1}\)). Species loss with N addition was modified by precipitation and sampling areas, being greater in wet years than dry years (Lan and Bai, 2012) but less at larger scale (Lan et al., 2015), suggesting climate/scale-dependent N impacts on biodiversity. Hao et al. (2018) reflected the cumulative and recoverable negative impacts of N addition on species richness in a semiarid temperate steppe, that is low N deposition/inputs could cause a significant species loss with N accumulation and further recycle over time. After the cessation of N addition (480 kg N ha\(^{-1}\) yr\(^{-1}\)), species richness rapidly recovered to and maintained 50-70% of that in the control plots with decreased concentrations of soil inorganic N and foliar N. Limited recovery from N addition might be due to soil acidification (soil pH decreased by ~1.5 units) and the absence of mowing. Further research found that mowing (removal of plant-N) could maintain diversity from N-induced species loss in the temperate steppe (Yang et al., 2019; Lu et al., 2021).
Eutrophication and acidification are two primary mechanisms driving plant species loss under enhanced N deposition. However, Tian et al. (2016) showed a soil manganese (Mn) related mechanism of N-induced species loss in a temperate steppe, where foliar Mn concentrations in forbs were 10 times higher than that in grasses following N-induced soil acidification and enhanced available Mn concentrations in soil, limiting the photosynthetic capacity of forbs.

Alpine grasslands in Asia, characterized by high solar radiation and low temperature with a large diurnal range, have received increasing attention to examine the N-impacts on species loss in the past decade, especially on Tibetan Plateau (Fu and Shen, 2016; Li et al., 2019; Wang et al., 2020). Yang et al. (2015) provided field evidence to confirm abundance- and functional-based mechanisms of N-induced species loss in an alpine meadow, while abundance-based mechanisms became more dominant if herbivores existed. However, plant species richness, as well as aboveground biomass, was observed to be not sensitive to N addition in an alpine grassland in the mid-Tianshan mountains of Central Asia (Li et al., 2015), which could be true if there are other resources limitations (e.g. water, temperature, or nutrients) or weak N-induced competition exclusions (light in especial) due to low species richness and density pressure. Similar weak species responses to N addition were also observed in the five-year N addition experiments in four alpine grasslands (alpine meadow, alpine meadow steppe, alpine steppe, and alpine desert-steppe, with a general low species richness (<10 species per m² in the control plots) along precipitation gradients on Tibetan Plateau (Zong et al., 2019). In contrast, significant species loss with N addition was observed in a Tibetan alpine meadow with higher species richness (~20 species per m²) (Niu et al., 2014), reflecting competitive exclusion as a mechanism of species loss.

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

Grasslands in Japan are distributed throughout subarctic-subtropic zones. Although N deposition in Japan is around the N critical loads for species loss, few studies have revealed the impacts of N deposition on species diversity (Lin et al., 2021), requiring further research.
Through many studies on a large spatial and temporal scale, there is a good understanding of species diversity in response to N deposition in grasslands in parts of Asia. With raised awareness of N pollution, implemented N emission controls and requirement for global carbon neutrality, N deposition will likely decrease in the near future, exactly as what Europe has experienced. However, there is a lack of understanding regarding ecological recovery of grasslands from N deposition in Asia. Many of the cited experiments use high levels of N addition. Significant soil acidification was observed in major northern China’s grasslands (Yang et al., 2012), this is an important mechanism of N-induced species loss and thus liming effects on species diversity are interesting to know in severe acidified grasslands. In addition, experimental N addition with care should be also considered to reduce the amplified N impacts in manipulative experiments, caused by N addition at high rates and low frequency (Zhang et al., 2014), to better predict species diversity in the future scenarios of N deposition.

6. Africa

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

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

Various long- and short-term studies have been carried out to determine the impacts of nutrient additions (including N) on grasslands and savanna composition, structure and productivity. These studies are mainly centred in southern and eastern Africa. While the levels of added N in these
studies may not necessarily approximate deposition levels, they nonetheless indicate trends of response to N addition, thus allowing for developing realistic models for assessing impacts of deposition.

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

These generalised responses have been confirmed in other studies in South Africa and further afield in Africa. Snyman and Oosthuizen (2001) recorded rapid changes in species composition from a climax state to a sub-climax state with three years of N application, with a concomitant increase in ANPP in a semi-arid grassland (MAP 560 mm). The authors also noted the role of P as a co-limiting nutrient. Craine et al. (2008) assessed the impacts of added N and P on ANPP across a rainfall gradient (MAP 457 – 737 mm) in the Kruger National Park in South Africa. They found a similar pattern across all five of their sites, with N addition increasing ANPP to a greater extent than P addition, but the combination of N and P showed the greatest increase in ANPP. Responses were unrelated to N and P levels and ratios in the vegetation. In an east African savanna (MAP 650 mm), ANPP responded in a similar manner to N and P addition in open savanna away from trees. Under leguminous tree canopies, where N fixation raised soil N levels, P emerged as the primary limiting
nutrient (Fulco et al., 2001). An experiment investigating the interaction of defoliation and N addition on a short grass from the Serengeti in east Africa showed a similar response of N addition boosting ANPP (William Hamilton III et al., 1998).

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

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

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

Lack of spatial representation of experiments across African grasslands and savannas inhibits comprehensive coverage of responses to added N. However, the convergence of impacts within
experiments in Africa and in comparative experiments with sites on other continents indicates a general convergence of responses.

7. Australasia

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

N deposition is not considered as a widespread threat to the biodiversity and integrity of Australian ecosystems, including grasslands and deposition is typically very low. The potential ecological consequences of climate change (Lindenmayer et al., 2010), land use change (Bell et al., 2014; Broadhurst and Coates, 2017), including urbanization and overgrazing, and species invasion of both plant and animal communities (Morgan, 1998; McIvor, 2005) of much greater concern. This lack of concern is reflected in the scarcity, almost completely absence, of scientific literature studying the potential impacts of N deposition on Australian grasslands, either in the form of experimental N addition studies simulating realistic doses, or in the form of observational studies across real gradients. For example, a recent meta-analysis of 174 publications evaluating N deposition in urban areas did not include any single study from Australia and the Pacific Islands (Decina et al., 2020).
is due to the fact that most of the country is devoid of human population, and population concentrations are very low across most of the country, which results in limited emissions of N compounds that are usually associated with large cities (Ayers et al., 1995; Dentener et al., 2006). Moreover, most of the big Australian cities are located upwind of polluting sources, resulting in an export of pollutants to the Pacific Ocean and its islands (Stohl et al., 2002; Longley et al., 2019). The general lack of water also limits the implementation of intensive agriculture across much of the territory, thus resulting in somewhat limited emissions of reduced N across the region. The most likely impacts of N deposition on Australian grasslands are thus expected to be linked to point sources (Ayers et al., 1995; Shen et al., 2016), including poultry, swine, and cattle farms, intensive agricultural operations. This is particularly the case across the most populated regions and the wheat belt, and also dust particles transported by the wind across long distances originating in China, Southeast Asia, and to a lesser extent, Africa (Stohl et al., 2002). Open-air mines are also another potential source of N to the atmosphere (Hendryx et al., 2020), but in this case the ecological impacts of N would be also associated with those of other polluting elements such as toxic metals. Anyhow, estimates of N deposition across Australian grasslands are very rare, and thus our best estimates are currently based on global models; current estimates are around 1-5 kg N ha\(^{-1}\) yr\(^{-1}\) (Dentener et al., 2006). Consistent with the low importance of N deposition in Australia, the rate of N creation across the last 60 years in Oceania is the lowest as compared to the rest of continents (Galloway et al., 2021).

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

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

8. Conclusions

It is clear from this review and other research that N deposition impacts on grasslands can be observed to greater or lesser extents across the world. Research effort into the impacts of nitrogen deposition on grasslands is not evenly distributed across the globe. We have a good understanding of impacts in some parts of the world, particularly Europe, North America and parts of Asia but much less understanding in other regions. In some areas, where deposition is low and likely to remain so into the future, this reflects the lack of pressure from N deposition as a driver of vegetation change but in other areas if reflects a lack of resources. Despite variation in our knowledge and understanding there are clear themes that emerge including around increased biomass production and reduction in plant species richness as N deposition increases. These common responses mean
that we can predict responses of grasslands in areas where we have little evidence of impacts. However, a research priority is identifying areas where N deposition is above critical loads used in other regions but impacts are not understood and identifying areas where N deposition is likely to increase and ensuring monitoring is in place (Table 1). In some regions, including parts of Europe and North America we are beginning to see declines in N deposition making understanding prospects for recovery an important area of research. Understanding interactions between N deposition and climate change is another important area where we currently have a number of knowledge gaps (Borer and Stevens, 2022).

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

<table>
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<th>Continent</th>
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| Europe       | • Changes in the ratio of reduced and oxidised N inputs  
              | • Recovery from N deposition                                                                         |
| North America| • Changes in the ratio of reduced and oxidised N inputs  
              | • Individual species responses                                                                        |
| South America| • Improved understanding of impacts of N on major grassland types  
              | • Understanding the role of N fixers and other plant soil interactions  
              | • Critical loads for major grassland types                                                         |
| Asia         | • Recovery from N deposition  
              | • Experiments with low N inputs and high frequency additions to refine predictions of community response |
| Africa       | • Improved understanding of impacts of N on major grassland types                                     |
| Australasia  | • Improved understanding of impacts of N on major grassland types  
              | • Interactions between N deposition and water availability and other nutrients                        |
Figure legends

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

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

**Graphical abstract**

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