

Plant traits of grass and legume species for flood resilience and N₂O mitigation

Natalie J. Oram^{1,2,3}  | Yan Sun¹  | Diego Abalos⁴  | Jan Willem van Groenigen¹  | Sue Hartley⁵  | Gerlinde B. De Deyn¹ 

¹Soil Biology Group, Wageningen University & Research, Wageningen, The Netherlands

²Department of Ecology, University of Innsbruck, Innsbruck, Austria

³Environmental Research Centre, Teagasc, Johnstown Castle Co, Wexford, Ireland

⁴Department of Agroecology, Aarhus University, Tjele, Denmark

⁵Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, UK

Correspondence

Natalie J. Oram

Email: natalie.oram@uibk.ac.at

Gerlinde B. De Deyn

Email: gerlinde.dedeyn@wur.nl

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Abstract

1. Flooding threatens the functioning of managed grasslands by decreasing primary productivity and increasing nitrogen losses, notably as the potent greenhouse gas nitrous oxide (N₂O). Sowing species with traits that promote flood resilience and mitigate flood-induced N₂O emissions within these grasslands could safeguard their productivity while mitigating nitrogen losses.
2. We tested how plant traits and resource acquisition strategies could predict flood resilience and N₂O emissions of 12 common grassland species (eight grasses and four legumes) grown in field soil in monocultures in a 14-week greenhouse experiment.
3. We found that grasses were more resistant to flooding while legumes recovered better. Resource-conservative grass species had higher resistance while resource-acquisitive grasses species recovered better. Resilient grass and legume species lowered cumulative N₂O emissions. Grasses with lower inherent leaf and root $\delta^{13}\text{C}$ (and legumes with lower root $\delta^{13}\text{C}$) lowered cumulative N₂O emissions during and after the flood.
4. Our results highlight the differing responses of grasses with contrasting resource acquisition strategies, and of legumes to flooding. Combining grasses and legumes based on their traits and resource acquisition strategies could increase the flood resilience of managed grasslands, and their capability to mitigate flood-induced N₂O emissions.

KEYWORDS

extreme weather event, flood, grassland, leaf traits, N₂O mitigation, recovery, resistance, root traits

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1 | INTRODUCTION

Ecosystems are facing increasing climatic challenges (Hazeleger et al., 2015), including more frequent and severe floods (IPCC, 2018). Flood events compromise ecosystem functioning by reducing primary productivity (Reyer et al., 2013), disrupting nitrogen (N) and carbon (C) cycling (Sánchez-Rodríguez et al., 2019) and may increase N losses via emissions of the potent greenhouse gas nitrous oxide (N_2O ; Gebremichael et al., 2017). In N-fertilized grasslands, flooding has been shown to drastically increase N_2O emissions compared to a non-flooded control (Oram, van Groenigen, et al., 2020). Determining if and how different plant species mitigate flood-induced N_2O emissions (Abalos et al., 2019) while maintaining primary productivity is an important management tool for creating resilient grasslands that mitigate N losses.

Previous research has shown the importance of plant traits for regulating N cycling and reducing N losses (Abalos et al., 2019; de Vries & Bardgett, 2016). Traits are indicative of a plant's strategy for nutrient uptake and growth; resource-acquisitive plants are fast growing with low tissue density and high rates of nutrient uptake, whereas slower-growing resource-conservative plants have opposite traits (Reich, 2014). A plant's resource acquisition strategy can also explain the rate of litter decomposition and N release (Roumet et al., 2016). Resource-conservative plants have been linked with greater N retention in extensively managed, non-fertilized grasslands (de Vries & Bardgett, 2012), while resource-acquisitive plants become more important to retain N in more intensively managed, fertilized grasslands (Wang et al., 2018). Acquisitive plants can take up more N than conservative species (Grassein et al., 2015) and reduce N losses by mitigating N_2O emissions (Abalos et al., 2018) and N leaching (de Vries & Bardgett, 2016).

Resource-conservative plants have been shown to have higher flood resistance than resource-acquisitive plants (Fischer et al., 2016) and could be important for mitigating N_2O in flood-prone grasslands. Species with stress-tolerant strategies, that is, a high leaf dry matter content (LDMC) and low specific leaf area (SLA), prevail in wet and/or flooded environments (Oddershede et al., 2018; Zelnik & Čarni, 2008). Higher survival of conservative plants in flooded environments could reduce N losses, as N uptake continues and N inputs via litter are lower, compared to less flood-tolerant species. Traits not considered in the acquisitive-conservative spectrum but related to plant stress responses could better predict plant flood resistance. For example, enrichment in leaf $\delta^{13}C$ relating to stomatal closure (Farquhar et al., 1989) and an increase in water use efficiency (WUE) (Mariotte et al., 2013) or formation of adventitious roots and root aerenchyma (Evans, 2004). Another potentially beneficial trait is enrichment in leaf silicon (Si), which improves cell water balance (Farooq et al., 2009) and is known to mitigate abiotic stress (Thorne et al., 2020).

Different strategies may be key to plant survival and growth at different points in the flooding cycle. A flood imposes stress through oxygen limitation when the soil is saturated (Bailey-Serres & Voesenek, 2008) and reductions in available N via leaching but

can also increase nutrient availability as the soil dries via decomposition of new organic matter, potentially benefiting plants that can survive the stress and capture these nutrients (Wright et al., 2015). Therefore, conservative plants may better cope with flooding stress, better *resisting*, whereas acquisitive plants could begin taking up nutrients as the soil dries, *recovering* faster. This trade-off has been observed in response to drought (Ingrisch et al., 2018), and could occur in response to flooding. This implies that plants with different resource strategies could mitigate N losses via N_2O emissions at different points during flooding and recovery.

Beyond a plant's inherent traits, the trait variation within a species could influence plant flood resilience and N retention. Such intraspecific variation can be as large as the difference between species in response to warming (Malyshev et al., 2016) or drought (Jung et al., 2014). Wetland plant species exhibit a high degree of intraspecific trait variation (Albert et al., 2011), responding to flooding by elongating their shoots, forming root aerenchyma, aquatic roots and leaves (Colmer & Voesenek, 2009), or reducing SLA (Poorter et al., 2009). These plastic responses allow plants to better tolerate oxygen stress (Voesenek & Bailey-Serres, 2015). However, it is not well known whether non-wetland plants exhibit intraspecific variation in response to flooding, and whether this relates with flood resilience and N_2O mitigation.

Designing flood-resilient grasslands that can mitigate flood-induced N_2O emissions requires a better understanding of grassland plant species flood responses, and the underlying role of plant traits and intraspecific trait variation. Here, we tested whether plant above- and below-ground traits (measured in non-flooded conditions) could predict a plant community's flood resilience and flood-induced N_2O emissions. We determined whether flood-induced intraspecific trait variation was related resilience and N_2O emissions in flooded conditions. We hypothesized that:

1. Resource-conservative species better resist flooding while resource-acquisitive species recover better.
2. Resource-conservative species reduce N_2O emissions in flooded conditions compared to resource-acquisitive species, but that this reverses after the flood has passed.
3. Flooding affects plant traits. Plant species that exhibit more intraspecific trait variation will be more flood resilient and reduce flood-induced N_2O emissions.

2 | MATERIALS AND METHODS

2.1 | Experimental design and setup

We established a 14-week greenhouse pot experiment (March–June 2019) with 12 grassland plant species grown in monocultures that experienced one of two rainfall treatments: temporarily flooded or non-flooded (control), replicated five times, arranged in five blocks: 12 plant species \times two rainfall treatments (flood or control) \times five

replicates = 120 pots/experimental units. The plant species covered a range of traits related to resource acquisition (Table S1): the grasses *Agrostis stolonifera*, *Alopecurus pratensis*, *Brachiaria humidicola*, *Dactylis glomerata*, *Festuca arundinacea*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*; and the legumes: *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus* and *Trifolium fragiferum*. The experimental timeline was as follows: sowing seeds—6-week establishment period (all 120 pots)—3-week flood ($n = 60$) or control ($n = 60$) treatment—5-week recovery ($n = 60$ pots that had experienced the flood) or control ($n = 60$). Greenhouse conditions were a day:night regime of 16:8 hr light:dark, 21:16°C.

Pots (20 cm diameter \times 38 cm depth, sealed bottom) were filled with 11 kg (dry weight equivalent) topsoil collected from a field (51°59'43.3"N, 5°39'17.6"E). The soil is a sandy loam (84% sand, 10% silt and 2% clay, with 4.1% organic matter, $\text{pH}_{\text{CaCl}_2}$ of 5.6) classified as a typic endoaquoll (Soil Survey Staff, 2014). Air-dry soil was mixed with demineralized water (0.14 g water/g dry soil, 60% water holding capacity, WHC). Granular calcium ammonium nitrate, CAN (30 kg N/ha), was mixed into the soil before potting.

Seeds (Barenbrug BV, the Netherlands) were surface sterilized (1:1 demineralized water: household bleach for 30 min), rinsed and sown. Pots were thinned to 100 individuals (40 individuals for *B. humidicola*, which had poor germination) 2 weeks after sowing.

Five weeks after sowing, above-ground biomass of all pots was cut 3 cm above the soil level, dried at 70°C for 72 hr and weighed. Immediately following cutting, monocultures were fertilized with CAN (100 kg N/ha), in line with managed grassland practices. Six weeks after sowing, pots in the flooded treatment were flooded with demineralized water, equivalent to 80 mm rainfall (100-year return, Rajczak et al., 2013), a realistic rainfall event within climate change scenarios (Stocker et al., 2013). The flooded water level was approximately 3 cm above the soil surface, below canopy height (i.e. no plants were submerged), and was maintained for 3 weeks. After, surface water was removed with a syringe and discarded. Pots in the control treatment were maintained at 60% WHC throughout the experiment by watering to weight 4–6 times per week. Directly after the 3-week flood (T1), and at the end of the experiment (T2, 5 weeks after the flood ended), above-ground biomass of all pots ($n = 120$) was harvested to 3 cm above the soil (as above). Root biomass was harvested at T2 by taking four soil cores (2 cm diameter \times 28 cm depth) per pot. The cores were pooled, stored in plastic bags at 4°C and washed over a 0.5-mm sieve. A representative subsample of roots was stored in 70% ethanol at 4°C for root morphology measurements. The rest of the clean roots were dried (70°C for 96 hr) and weighed.

2.2 | Plant traits

Leaf traits of all monocultures ($n = 120$) were measured at T1 and T2. SLA and LDMC were measured according to Pérez-Harguindeguy et al. (2016), see Table 1 for trait acronyms and units. Above-ground biomass harvested at T1 was analysed for percent P, K, S, Ca and Si

TABLE 1 List of plant traits measured, their abbreviations and units

Abbreviation	Plant trait	Unit
Leaf traits		
SLA	Specific leaf area	cm ² /g
LDMC	Leaf dry matter content	mg/g
Leaf N	Leaf nitrogen concentration	mg N/g dry leaf
Leaf C	Leaf carbon concentration	mg C/g dry leaf
Leaf $\delta^{13}\text{C}$	Leaf natural abundance ¹³ C	‰
Leaf $\delta^{15}\text{N}$	Leaf natural abundance ¹⁵ N	‰
N _{dfa}	Nitrogen derived from the atmosphere	%
Leaf P	Leaf phosphorus concentration	%
Leaf K	Leaf potassium concentration	%
Leaf Ca	Leaf calcium concentration	%
Leaf S	Leaf sulphur concentration	%
Leaf Si	Leaf silicon concentration	%
Root traits		
SRL	Specific root length	m/g
RTD	Root tissue density	g/cm ³
Root N	Root nitrogen concentration	mg N/g dry root
Root C	Root carbon concentration	mg C/g dry root
Root $\delta^{13}\text{C}$	Root natural abundance ¹³ C	‰
Root $\delta^{15}\text{N}$	Root natural abundance ¹⁵ N	‰

by ball-milling dried leaves and pressing into pellets using a hydraulic press. Pellets were analysed with a portable P-XRF instrument (Niton XL3t900 GOLDD Analyzer; Thermo Scientific; Reidingen et al., 2012). Above-ground biomass (T1), and above- and below-ground biomass (T2), was analysed for N, C and natural abundance $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Leaf or root material was ball-milled, weighed into tin cups (D1008; Elemental Microanalysis, UK) and analysed using an elemental analyser (PDZ Europa ANCA-GSL interfaced to a PDZ Europa 20-20 IRMS; Sercon Ltd.). Natural abundance $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are expressed in ‰ deviation from reference standard pee-dee belemnite (¹³C), respectively, from air-N₂ (¹⁵N). Biological N fixation (N_{dfa}) was estimated by growing legumes in N-free sand (see Supplementary Methods).

A subsample of fresh roots were stained with neutral red (1.54 g/L demineralized water), scanned (Epson Perfection V700/750), root length and volume were estimated with WinRhizo (Regent Instruments Inc., Canada), and scanned roots were dried (70°C for 48 hr).

Traits measured on plants in the control treatment were used to predict the resistance, recovery and N₂O emissions from monocultures in the flooded treatment. Flood-induced intraspecific trait variation (the relative flood effect) was calculated in line with de Vries et al. (2016):

$$\text{Relative flood effect} = \frac{\text{Flood trait species } j, \text{ block } i - \text{Control trait species } j, \text{ block } i}{\text{Control trait species } j, \text{ block } i} \quad (1)$$

Leaf N uptake was calculated by multiplying above-ground biomass (g/pot) by leaf N concentration (mg N/g leaf) measured at T1 or T2. Root N uptake is root N concentration at T2 multiplied by root biomass accumulated over the entire experiment.

2.3 | Nitrous oxide emissions

Fluxes of N₂O were measured 29 times over 14 weeks using the closed flux chamber technique. Like Abalos et al. (2014), flux measurements were taken daily for 4 days after fertilizer addition and flooding, and subsequently two to three times per week. Polypropylene flux chambers (20 cm diameter × 14 cm height) fitted with Teflon tubes were placed on the pots for approximately 30 min. Gas measurements of the headspace were taken with an Innova 1312 photo-acoustic infrared gas analyser (LumaSense Technologies A/S). Cumulative emissions were calculated assuming linear changes in gas concentration between measurements, which has been previously tested with this experimental setup (Abalos et al., 2014, 2018).

2.4 | Calculations and statistical analysis

Flood resistance and recovery were calculated in line with Ingrisch and Bahn (2018), using control monoliths as a dynamic baseline, paired by plant species within a block. Resistance is the baseline normalized above-ground biomass of the flooded pots directly after the flood, and recovery is after a 5-week recovery period:

$$\text{Resistance or recovery (\%)} = \frac{\text{Flood aboveground biomass species } j, \text{ block } i}{\text{Control aboveground biomass species } j, \text{ block } i} \times 100 \quad (2)$$

Statistical analyses were performed in R 3.6.2. (R Core Team, 2019). Two high outliers were removed from analysis of cumulative N₂O emissions, one which was mistakenly over-thinned at the start of the experiment (*P. pratense*, flood) and one that was mistakenly over-fertilized (*P. pratense*, control). One above-ground biomass sample (*T. pratense*, flood, T2) went missing before it could be analysed for C, N, δ¹³C and δ¹⁵N; thus, it is not included in the analyses of these factors. Finally, six flooded monocultures had exceptionally high cumulative N₂O emissions (four *P. pratense* and two *P. pratensis*), relationships were tested with and without these six points, and both results are reported.

We tested the effects of plant species (12) or functional group (grass or legume) and rainfall treatment (control or flood) and the two-way interactions on cumulative N₂O emissions (over the entire experiment), above-ground biomass (sum of three harvests), below-ground biomass and plant traits using linear mixed-effects models, lme{nlme} (Pinheiro et al., 2016), with block as a random factor, fitted with a type III SS ANOVA, anova{base}. Tukey post-hoc tests were used to determine significance between levels of a factor using emmeans{emmeans} (Lenth, 2018).

A plant species' resource acquisition strategy was determined with principle component analysis (PCA) using the function pca{FactoMineR} (Le et al., 2008) on scaled (mean 0, SD ±1) traits indicative of the leaf or root economic spectrum at T1 (leaf) and T2 (leaf and root). The relationships between control plant traits or above-ground resource strategy (PCA 1), or the relative flood effect (Equation 1), and resistance, recovery, cumulative N₂O emissions until the end of the flood (T0–T1) or during the recovery period (T1–T2) were tested with lme models and ANOVA (as above). Relations frequently differed between grasses and legumes, so functional group and its interaction with the trait/strategy were included as explanatory variables. We further tested relations separately for legumes (*n* = 20) and grasses (*n* = 40) to understand relations within each functional group. N₂O emissions (T1–T2) were log₁₀ transformed to improve model fit. Residual plots were used to determine normality and homogeneity of variances. When residual variances were heterogeneous a variance structure was included in the model, varIdent{nlme}. Models with the variance structure were retained when they had a significantly lower AIC than the model without (tested with ANOVA).

3 | RESULTS

3.1 | Plant species flood resistance and recovery

Plant species differed in their flood resistance ($F_{11,44} = 22.90$, $p < 0.001$) and recovery ($F_{11,44} = 15.93$, $p < 0.001$), Figure 1. Legumes had lower resistance than grasses ($F_{1,54} = 61.19$, $p < 0.001$), but higher recovery ($F_{1,54} = 4.12$, $p < 0.05$). Flooding reduced the above-ground biomass of most species (Figure S1a). Grasses produced more root biomass than legumes (Figure S1b).

3.2 | Plant traits help predict plant species resistance and recovery

Leaf traits followed the leaf economic spectrum (Figure 2A,B). Root traits did not vary along the root economic spectrum as predicted, primarily due to the strong impact of the legumes on root N, and the root economic spectrum was not used in further analysis (Figure 2C).

Traits of plants grown in control conditions helped predict monoculture flood resistance and recovery. Conservative grasses (low PCA 1 value, low SLA, leaf N and high leaf C:N) were more flood resistant (Figure 3, for statistics, see Table S2). High grass resistance was also related with lower LDMC, leaf δ¹⁵N, leaf and root δ¹³C, and SRL (Figure 3). Legumes with lower leaf and root C, root δ¹⁵N and N_{dft} had higher flood resistance while resistance increased with increasing leaf δ¹⁵N and leaf P (Figure 3).

Acquisitive grasses (a high PCA 1 value, SLA, leaf N and low leaf C:N) had higher recovery after 5 weeks than conservative grasses (Figure 4, for statistics, see Table S2). Grass recovery decreased with increasing leaf C, leaf and root δ¹³C, and leaf δ¹⁵N (Figure 3). Grass recovery was positively related with SRL until 400 m/g and

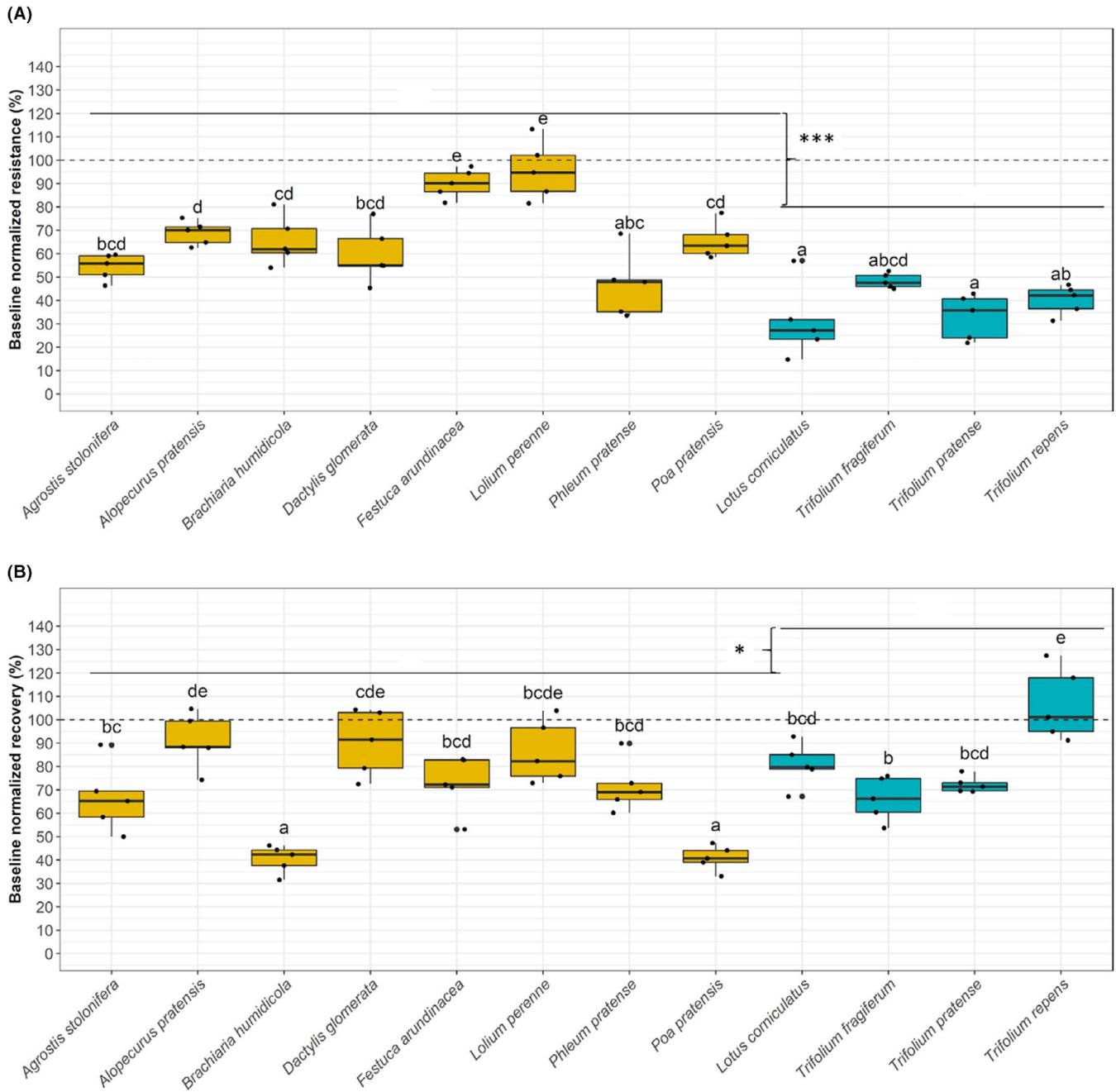


FIGURE 1 (A) Resistance, measured directly after the 3-week flood, and (B) recovery, measured 5 weeks after the flood ended, of grasses (yellow boxes) and legumes (blue boxes). Letters indicate significant differences between species, stars indicate differences between grasses and legumes based on a Tukey post-hoc test. The dashed line indicates the baseline

negatively related at higher SRL values while legume recovery was positively related with SRL (Figure 4).

3.3 | Monocultures differed in cumulative N₂O emissions

Flooding significantly increased cumulative N₂O emissions over the entire experimental period; a 3–288 fold increase depending on plant species (including the six high grass N₂O values, flooding: plant species interaction, $F_{11,90} = 44.98, p < 0.001$, Figure 5). In the flooded

treatment, N₂O emissions from legume monocultures were higher than from grass monocultures (including the six high grass N₂O values, $F_{1,53} = 305.86, p < 0.001$). In control conditions, cumulative N₂O emissions did not differ between legume and grass monocultures. Fluxes of N₂O peaked at the onset and recession of the flood (Figure S2).

3.4 | What mediates flood-induced N₂O emissions?

Cumulative N₂O emissions decreased with increasing plant biomass above- (the sum of three harvests; $F_{1,42} = 643.19, p < 0.001$)

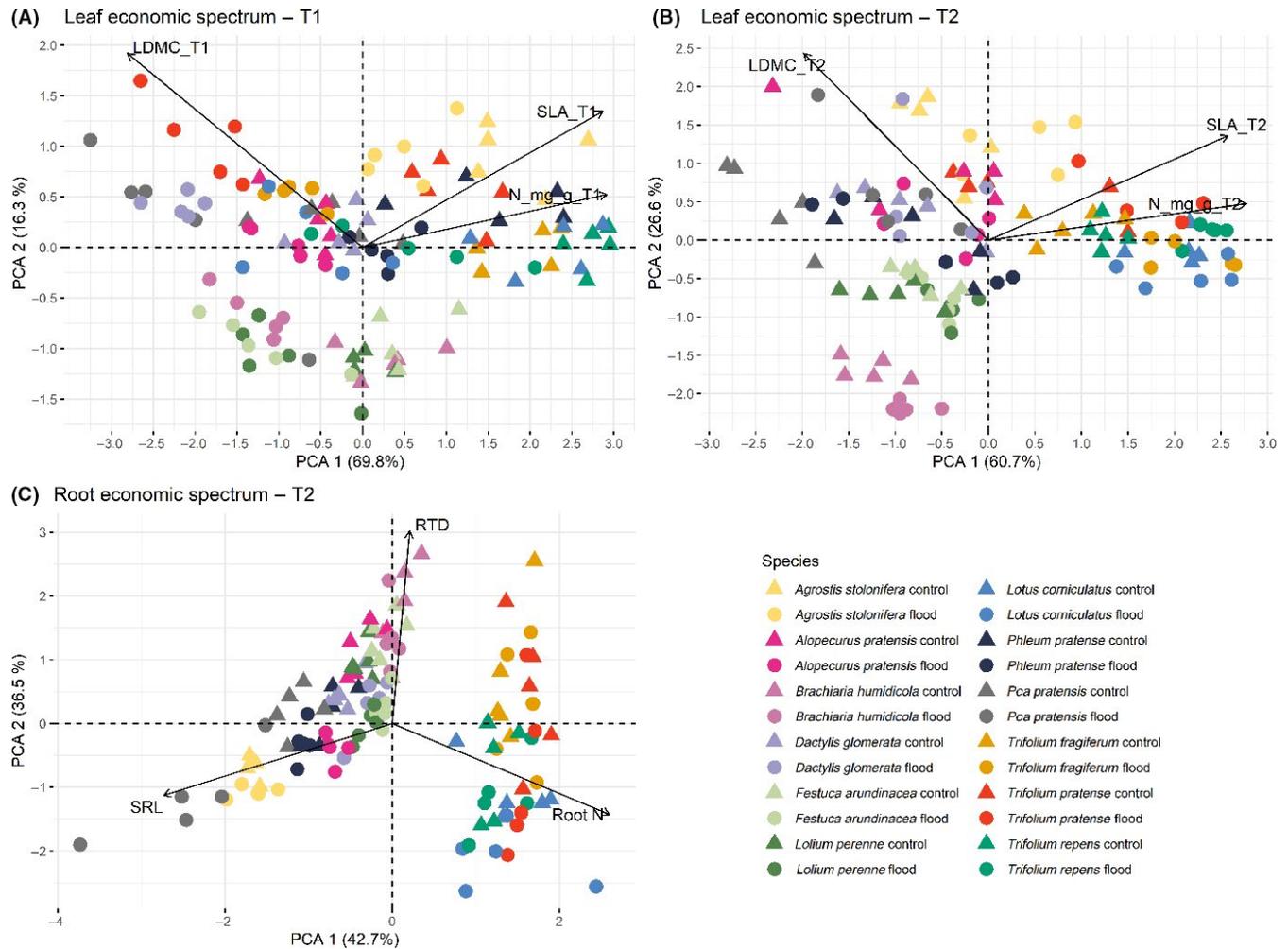


FIGURE 2 PCA biplots of the leaf economic spectrum (A) directly after flooding (T1) and (B) after the recovery period (T2) and the PCA biplot of (C) the root economic spectrum (T2)

and below-ground ($F_{1,42} = 1,388$, $p < 0.001$), and these relations remained significant without the six high N_2O values (above-ground: $F_{1,37} = 16.75$, $p < 0.001$, below-ground: $F_{1,47} = 315.75$, $p < 0.001$). Higher grass leaf N uptake (T1) was related with lower N_2O emissions until the end of the flood (T0–T1, $F_{1,33} = 24.64$, $p < 0.001$). During the recovery, this relation reversed as grasses with high N uptake (T2) related with higher N_2O emissions (T1–T2, $F_{1,28} = 6.23$, $p < 0.05$; removal of the six high points did not change significance). Legume leaf N uptake did not relate with N_2O emissions during the flood (T0–T1) but was negatively related to N_2O emissions during recovery (T1–T2, $F_{1,13} = 55.18$, $p < 0.001$). Below-ground, grass root N uptake was negatively related with cumulative N_2O emissions during the recovery period (T1–T2, $F_{1,28} = 24.15$, $p < 0.001$). Monocultures with higher resistance emitted less N_2O during the flood (T0–T1, $F_{1,31} = 16.61$, $p < 0.001$, Figure S3a), and higher recovery was related with lower N_2O emissions afterwards (T1–T2, $F_{1,27} = 136.11$, $p < 0.001$, Figure S3b).

Grass monocultures with higher leaf C:N and lower leaf and root $\delta^{13}C$ emitted less N_2O emissions T0–T1 (Figure 6). Legume monocultures with higher LDMC, leaf C:N, N_{dfa} , leaf Ca, root C, and root $\delta^{13}C$,

and lower leaf $\delta^{15}N$, leaf Si, leaf K, and leaf S emitted less N_2O during this period (Figure 6, for statistics, see Table S3).

Acquisitive grasses (a high PCA 1, Figure 1A) emitted less N_2O than conservative ones during the recovery, T1–T2 (Figure 7A). Excluding the six high outliers, N_2O emission from grass monocultures during recovery were positively related with leaf C:N (opposite to the relation during T0–T1), leaf $\delta^{13}C$, leaf $\delta^{15}N$, root N and root $\delta^{13}C$, and negatively related with leaf N and root C:N (Figure 7). Cumulative N_2O from legume monocultures was negatively related with N_{dfa} and SRL (Figure 7, for statistics, see Table S3).

3.5 | Flood-induced intraspecific trait variation

Leaf traits measured after the flood (T1) were distinctly grouped in flooded or control treatments (Figure S4a). Traits related to tissue strength and stress tolerance were grouped, and negatively related with traits related to higher resource uptake (Figure S4a). The change in some traits differed between grasses and legumes (treatment: functional group interaction; Figure S5; Table S4) and between

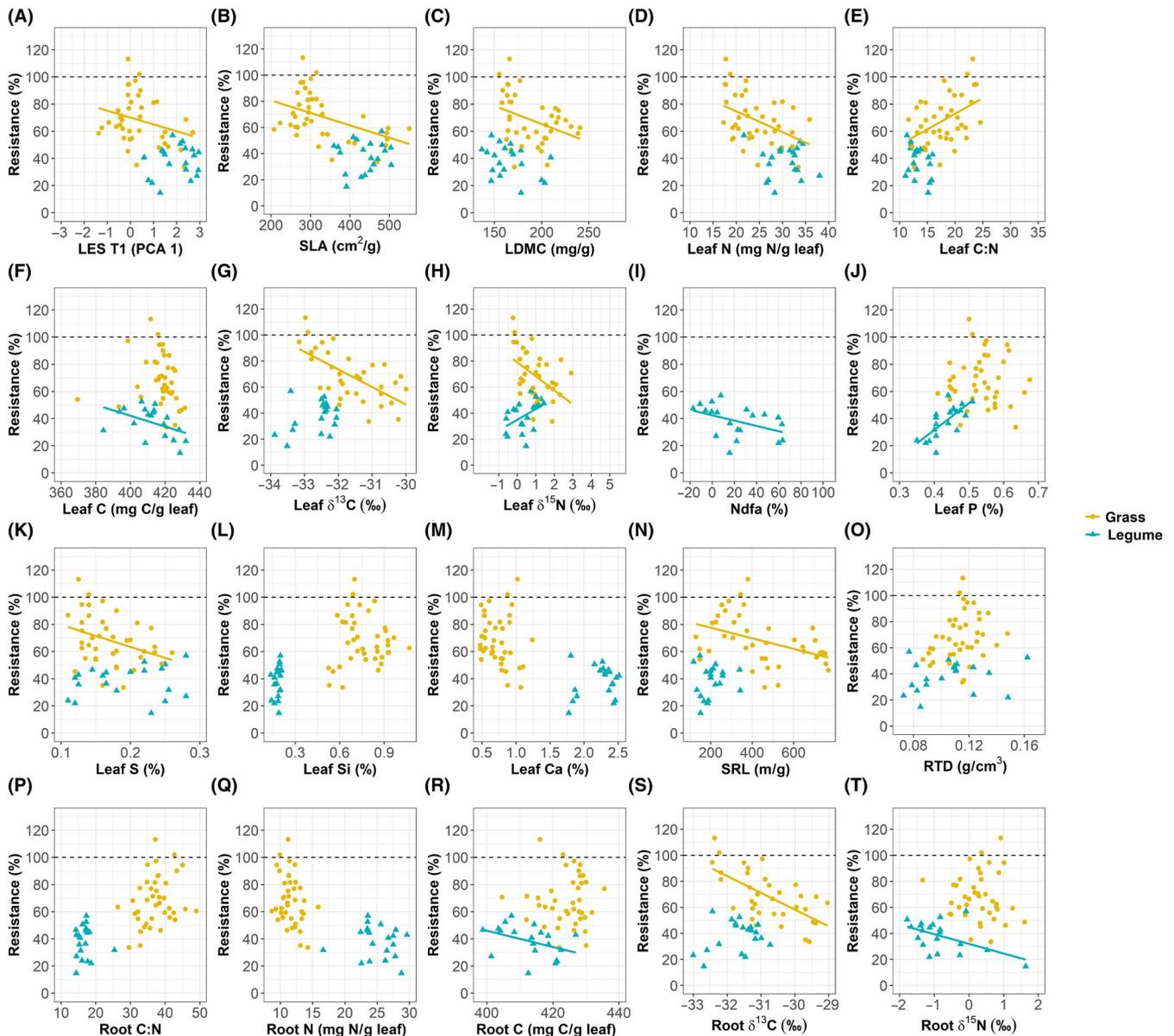


FIGURE 3 (A–T) Resistance relations with plant traits (measured in control conditions). Lines indicate significant relationship within grasses (yellow, $n = 40$) or legumes (blue, $n = 20$). One low point (*Lotus corniculatus*) was removed from (F), which did not change the relation. *Brachiaria humidicola* was removed from G, S, as it is a C4 species. For acronyms, see Table 1, for statistics, see Table S2

species (treatment: species interaction; Table S4). During the recovery period, legume SLA increased and LDMC decreased in previously flooded monocultures compared to the control (Figure S5b). Previously flooded grass and legume monocultures became more acquisitive compared to the control (Figure S5b). All other traits did not differ between previously flooded or control monocultures (Figure S5b), and traits were no longer grouped according to flood or control treatment (Figure S4b).

Flood-induced shifts in plant traits were not closely related to increases in resistance or recovery (Figure S6). Lower flood-induced N_2O emissions T0–T1 were related with reductions in grass leaf $\delta^{13}C$ (Figure S7). Legume monocultures that reduced leaf P, K and Ca emitted less N_2O T0–T1 (Figure S7). After the flood, only grass leaf and root $\delta^{15}N$ were positively related with N_2O

emissions, likely resulting from enrichment in soil $\delta^{15}N$ with greater denitrification.

4 | DISCUSSION

We investigated whether plant traits and plant strategies could predict flood resilience and flood-induced N_2O emissions. Plant traits related differently to flood resistance than to recovery, indicating that different plant strategies are necessary for survival and regrowth, similar to drought studies (Ingrisch et al., 2018; Volaire et al., 2014). Conservative grasses better resisted flooding while acquisitive grasses recovered better. Legumes had lower flood resistance but higher flood recovery than grasses, which was not

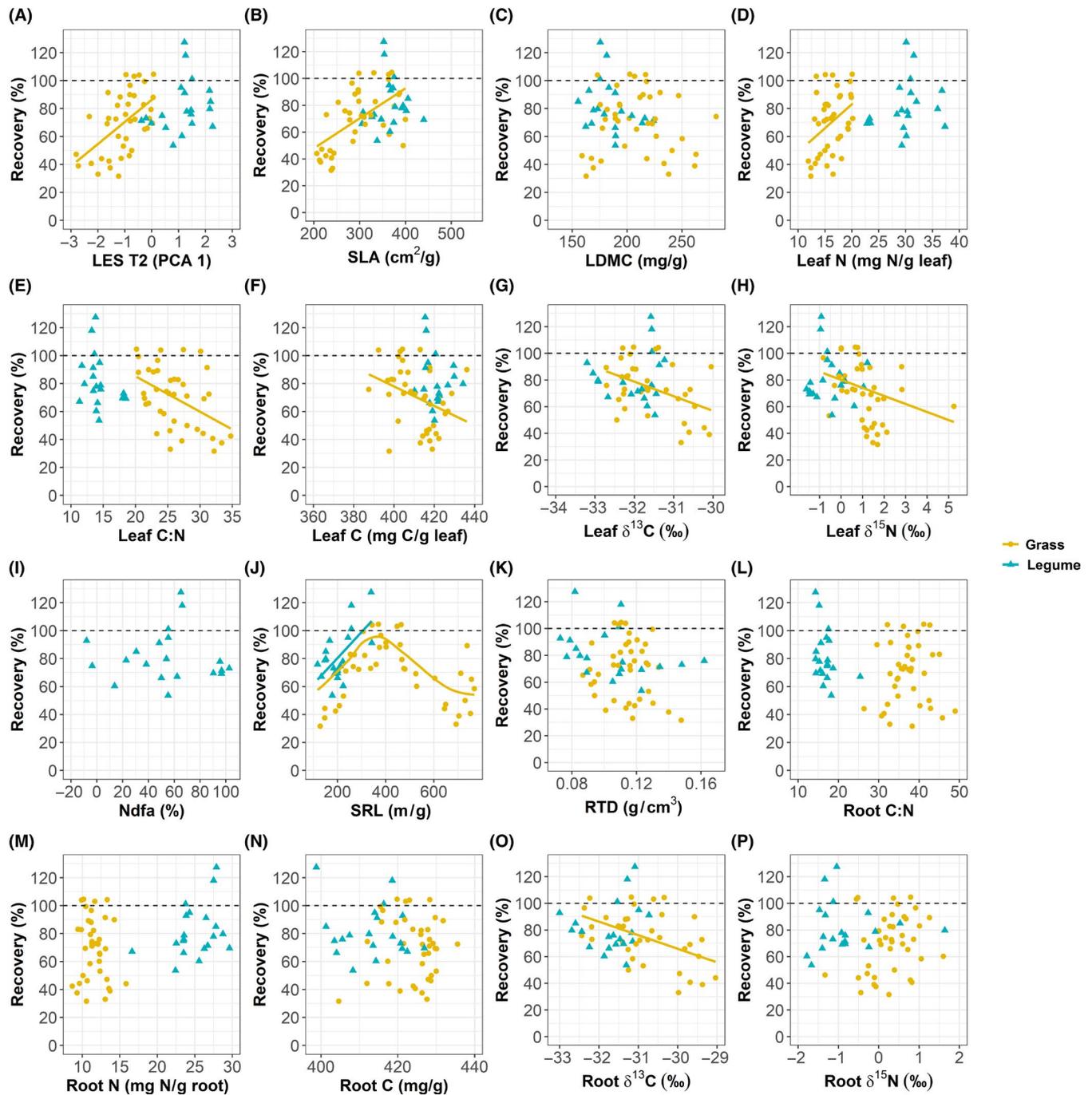


FIGURE 4 (A–P) Recovery relations with plant traits (measured in control conditions). Lines indicate significant relationship within grasses (yellow, $n = 40$) or legumes (blue, $n = 20$). *Brachiaria humidicola* was removed from G, O, as it is a C4 species. For acronyms, see Table 1, for statistics, see Table S2

related to their resource acquisition strategy. High resistance and recovery lowered cumulative N_2O emissions regardless of the plant functional group, indicating that survival during and regrowth after a flood can mitigate N_2O emissions. Identifying the underlying role of plant traits in grassland flood resilience and N_2O emissions is a step towards designing flood-resilient managed grasslands that mitigate N_2O . We considered the early growth stages of perennial grassland species and flooding in summer conditions. Longer-term studies that follow trait relations with resilience and N_2O over different seasons

are needed to increase our capacity to predict how managed grasslands will respond to flooding.

4.1 | Plant functional group and flood resilience

Grasses were more resistant than legumes, consistent with previous research on flooding (Ploschuk et al., 2017) and drought (De Boeck et al., 2018). In contrast, legumes recovered better than grasses 5

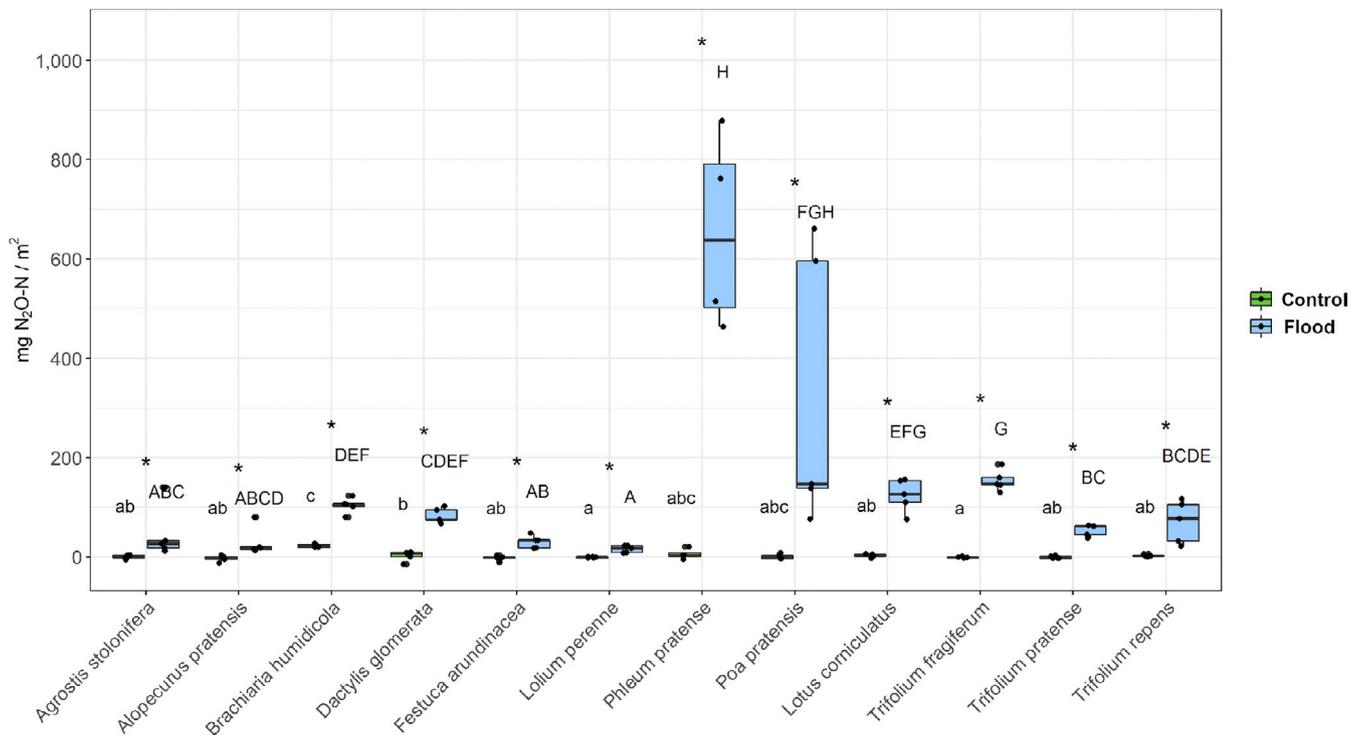


FIGURE 5 Cumulative N₂O emissions in flooded and control conditions. Significant differences between monocultures are indicated by a small letter (non-flooded/control), or a capital letter (flood). Differences between flood and control within a plant species are indicated with a star

weeks after flooding, in line with Hofer et al. (2017) who found that legumes recovered quickly after drought. Thus, low resistance does not preclude fast recovery (Striker, 2012), and both resistance and recovery should be considered when selecting species for flood-resilient grasslands. The legumes *T. pratense* and *T. repens* produced the highest cumulative above-ground biomass in our experiment, aligning with the goal of sustaining high primary productivity in managed grasslands. Legumes are a vital component of managed grasslands as they can transfer biologically fixed N to surrounding non-legumes (Thilakarathna et al., 2016), promote C and N retention (De Deyn et al., 2009), and reduce the need for fertilizer while maintaining grassland yield (Fuchs et al., 2018). In combination with resistant grasses (e.g. *L. perenne* or *F. arundinacea*), legumes with high recovery could increase grassland yield stability in a climate with frequent floods.

4.2 | Plant traits and flood resistance and recovery

Resource-conservative grasses were more flood resistant while resource-acquisitive grasses had higher recovery after 5 weeks, in line with responses to drought (Ingrisch et al., 2018). Frequently flooded grasslands were found to favour conservative species with low SLA and high LDMC (Moor et al., 2017), and in a managed grassland, more conservative plant communities were found to better resist repeated flooding (Oram, De Deyn, et al., 2020). After the stress has passed, acquisitive species could benefit from flood-induced increases in nutrient availability (Wright et al., 2015), resulting in their fast recovery.

We found that other traits (measured in control conditions) not included in the resource acquisition strategy gradient could explain plant species resistance and recovery, and these relations generally differed between grasses and legumes. Grasses with a lower leaf and root $\delta^{13}\text{C}$ in control conditions were more resistant to flooding. Leaf $\delta^{13}\text{C}$ has been shown to decrease with increasing mean annual precipitation, indicating lower WUE (Diefendorf et al., 2010). Thus, plant species with a lower $\delta^{13}\text{C}$ in control conditions may be better suited to wet environments, as they could keep their stomata open for longer. However, without knowing stomatal conductance, or proxies such as $\delta^{18}\text{O}$ (Scheidegger et al., 2000), the extent to which the relation between inherent $\delta^{13}\text{C}$ and resistance or recovery can be interpreted in terms of plant physiology is limited. The strong $\delta^{13}\text{C}$ -resistance/recovery relations we find require further research to understand the underlying mechanisms and determine if inherent leaf and root $\delta^{13}\text{C}$ can be reliably used to predict plant species flood resilience. Grasses with thicker roots had higher resistance, potentially because they are less susceptible to damage and radial oxygen loss, promoting oxygen diffusion along the roots and allowing them to function in flooded conditions (Pedersen et al., 2020). Grasses with low leaf C:N, and leaf and root $\delta^{13}\text{C}$ had higher recovery within 5 weeks. These traits align with an acquisitive strategy, indicating potentially higher transpiration and faster N uptake.

We found that legumes with higher leaf P concentration better resisted the flood, in line with Striker and Colmer (2017). This signals that legumes with the capacity for higher P uptake (via characteristics in root architecture, exudation or association with mycorrhizal fungi) may be better equipped to deal with flooding stress. Legumes

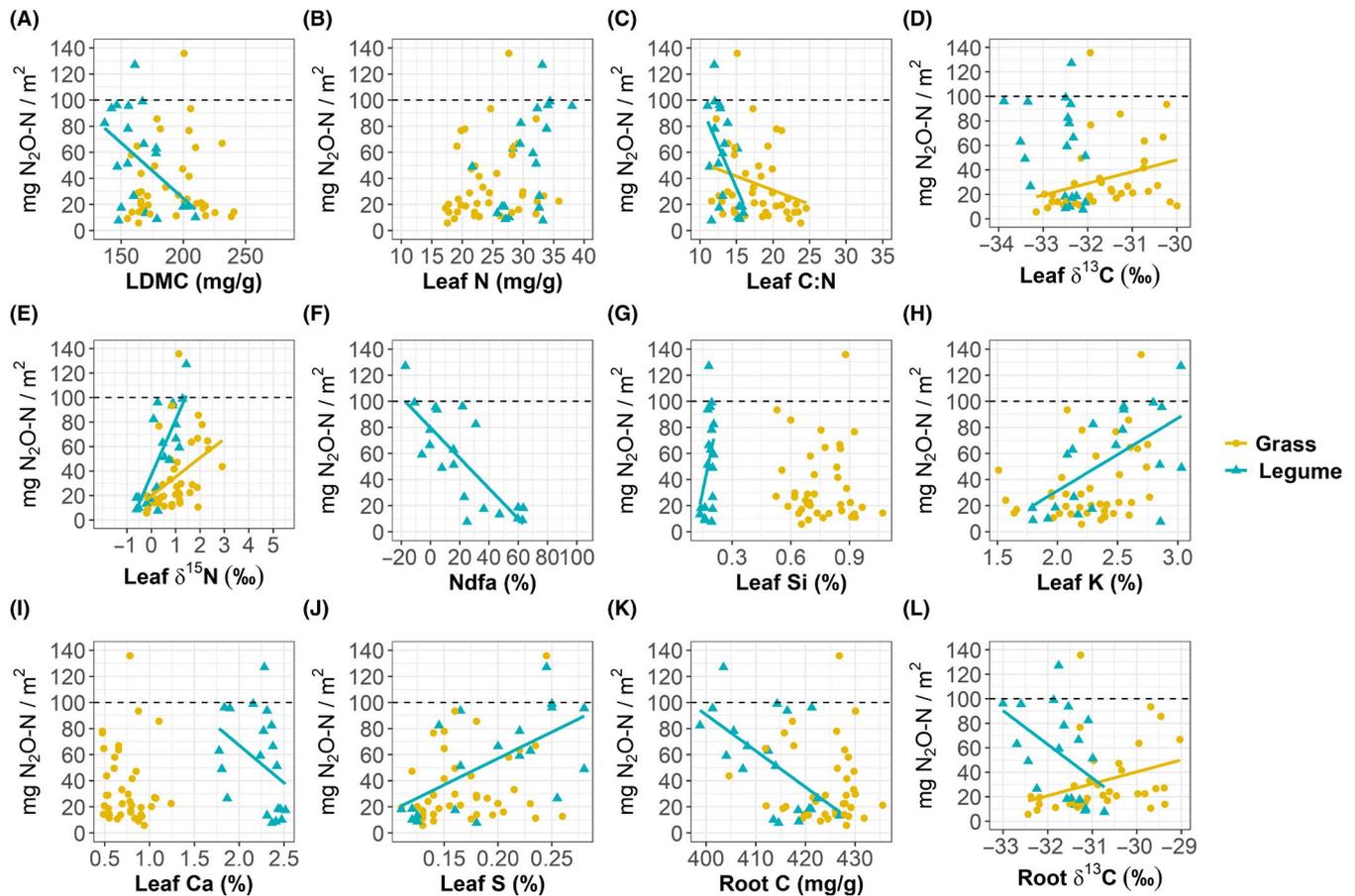


FIGURE 6 (A–L) N_2O emissions from flooded monocultures (T0–T1) were related with traits (measured in the controls). Lines indicate significant relationship within grasses (yellow, $n = 40$) or legumes (blue, $n = 20$). For acronyms, see Table 1, for statistics, see Table S3

with a higher N_{dfa} in control conditions were less resistant to flooding, perhaps because legumes with higher symbiont dependence to meet their N nutrition suffer more when this symbiosis is potentially disrupted by flooding (Striker & Colmer, 2017). Legumes with higher SRL had higher recovery 5 weeks after flooding, possibly because legumes with fine roots are better able to take up nutrients post-flood, improving recovery.

4.3 | Flood-induced N_2O emissions

Flooding drastically increased N_2O emissions, consistent with previous studies (Oram, van Groenigen, et al., 2020; Sánchez-Rodríguez et al., 2019). Plant species with a higher resistance and recovery mitigated flood-induced N_2O emissions. Plant species with lower flood resilience could increase N_2O emissions via dead plant litter that can be mineralized, as well as decreases in N uptake, both resulting in higher N availability for nitrifiers and denitrifiers. Plant species that recovered quickly likely mitigated flood-induced N_2O emissions by taking up N, which is supported by the negative relation between root N uptake and cumulative N_2O , as well as by increasing soil dry down and reducing soil water content.

Cumulative N_2O emissions were only related with grass resource acquisition strategy in the recovery phase, and not during the flood,

in contrast to our second hypothesis. Plant species with a high leaf C:N ratio had lower N_2O emissions until the end of the flood, perhaps because N-poor leaf litter is slower decomposing than N-rich litter (Roumet et al., 2016), and slower decomposition could underlie a negative relation between C:N ratio and N_2O emissions in flooded conditions (Oram, van Groenigen, et al., 2020). Legume LDMC was negatively related with N_2O emissions until the end of the flood potentially also because of slower decomposition (Freschet et al., 2012). Post-flooding, resource-acquisitive grasses (high SLA and leaf N) had lower N_2O emissions than conservative grasses. Acquisitive grasses also had higher recovery 5-week post-flood, which was related to lower N_2O emissions. This is broadly in line with studies reporting that acquisitive, productive grasses can mitigate N_2O emissions in non-flooded conditions (Abalos et al., 2018, 2020).

4.4 | Flood-induced intraspecific trait variation

We found that plant species subjected to flooding became more resource conservative, by increasing LDMC and reducing leaf N concentration, similar to studies showing that conservative species become more dominant in flooded environments (Moor et al., 2017; Oddershede et al., 2018). Flooding reduced grass leaf $\delta^{13}\text{C}$, indicating lower WUE (Farquhar et al., 1989), which signals that grasses were

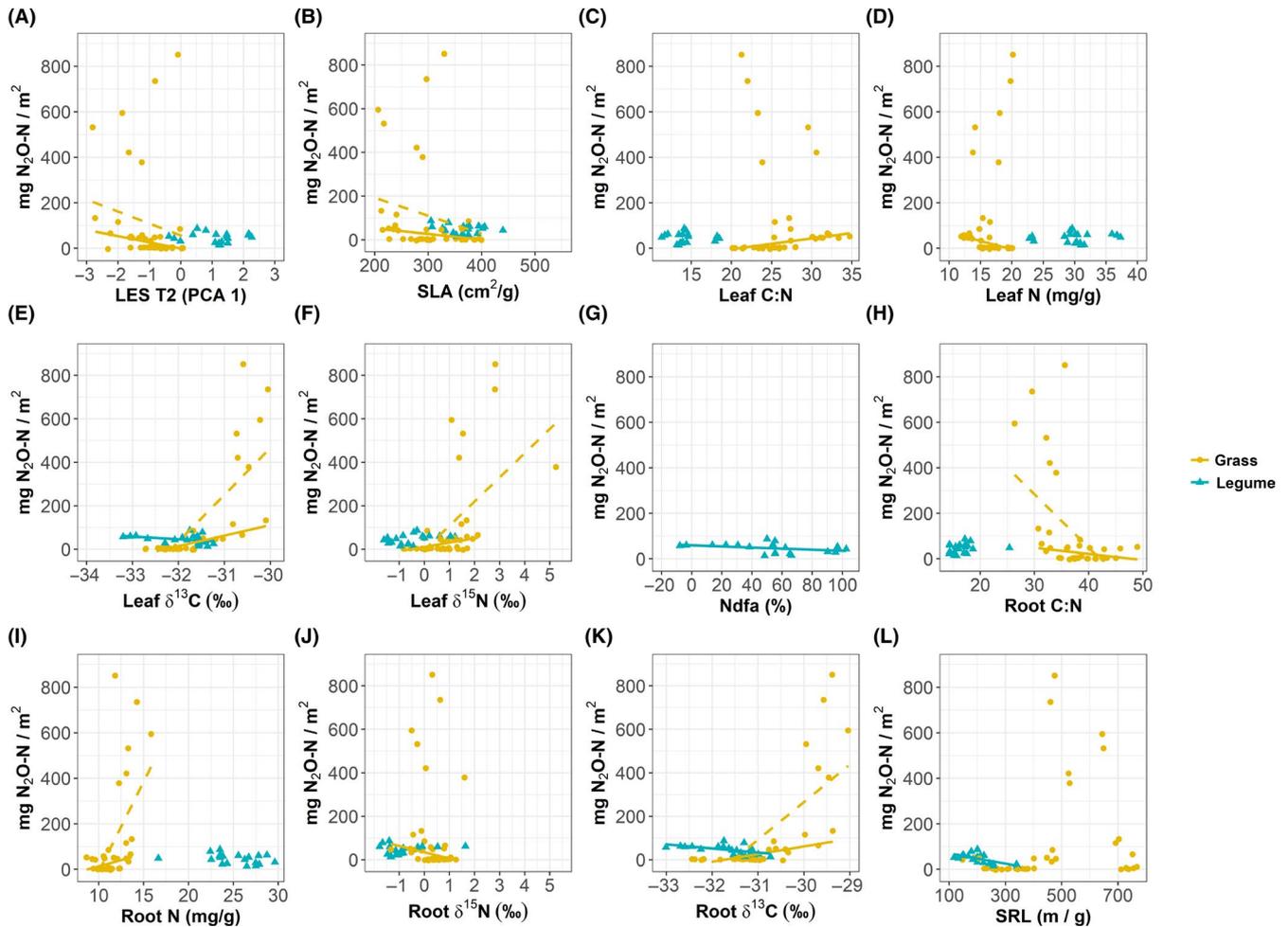


FIGURE 7 (A–L) N_2O emissions from flooded monocultures (T1–T2) were related with traits (measured in the controls). Lines indicate significant relations; dashed lines include the six high points ($n = 39$), solid lines exclude these points ($n = 33$) for grasses (yellow) and legumes (blue, $n = 20$). For acronyms, see Table 1, for statistics, see Table S3

able to take up water and increase transpiration during the flood. In contrast, the legumes *L. corniculatus* and *T. pratense* increased their leaf $\delta^{13}C$ (and thus WUE), a response frequently reported from drought studies (e.g. Mariotte et al., 2013). Flooding generally decreased leaf nutrient concentrations, but increased legume leaf Si. Silicon uptake has been linked to plant drought stress response, and can alleviate stress by increasing antioxidant enzymes and reducing oxidative damage (Thorne et al., 2020). Effects of Si on transpiration rates are variable, and uptake of Si in the transpiration stream is itself also altered by water availability (Brightly et al., 2020). The differing responses of leaf Si concentration between grasses and legumes potentially reflect the lower flood resistance of legumes compared to grasses: the increase in legume leaf Si could be a response to this greater stress as Si can alleviate abiotic stress in legumes (Putra et al., 2020). Five weeks after flooding, grasses and legumes became more acquisitive, increasing their SLA and reducing LDMC, compared to the same species that did not experience the flood. SLA can increase with growing season precipitation (Dwyer et al., 2014) and soil fertility (Ordoñez et al., 2009). The increase in N availability after flooding, supported by our finding that root N

concentration of flooded plants was higher than non-flooded plants, could contribute to the shift in plant strategy.

4.5 | Flood-induced intraspecific variation in traits were not good predictors of resilience or N_2O emissions

In contrast to our hypothesis, flood-induced trait shifts were not generally related to higher resistance or recovery except for grass SLA, which was reduced in response to the flood and related to increased flood resistance. This could indicate a flood-tolerating strategy (Moor et al., 2017), or a response to lower N availability during the flood, as grass SLA generally decreases with decreasing soil N (Knops & Reinhart, 2000). Flooding reduced leaf N concentration of all grasses, and the greater the decrease, the lower the resistance. Thus, the more flooding compromised N uptake, the less the species were able to resist. Overall, flood-induced intraspecific trait variation was not a good predictor of resilience or flood-induced N_2O emissions.

5 | CONCLUSIONS

Flooding reduced plant biomass production and substantially increased N₂O emissions. Resource-conservative grasses were more flood resistant, whereas resource-acquisitive grasses recovered better. More resilient plant species mitigated flood-induced N₂O emissions. Thus, combining grasses with different resource acquisition strategies and legumes could improve grassland flood resilience and mitigate flood-induced N₂O emissions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

N.J.O., D.A., J.W.v.G. and G.B.D.D. designed the experiment; N.J.O. and Y.S. conducted the experiment and analysed the data; N.J.O. wrote the manuscript with input from Y.S., D.A., J.W.v.G., S.H. and G.B.D.D.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.59zw3r27h> (Oram et al., 2021).

ORCID

Natalie J. Oram  <https://orcid.org/0000-0002-3529-5166>

Yan Sun  <https://orcid.org/0000-0003-2313-1719>

Diego Abalos  <https://orcid.org/0000-0002-4189-5563>

Jan Willem van Groenigen  <https://orcid.org/0000-0001-9637-0601>

Sue Hartley  <https://orcid.org/0000-0002-5117-687X>

Gerlinde B. De Deyn  <https://orcid.org/0000-0003-4823-6912>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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