# Plants are less negatively affected by flooding when growing in species-rich plant communities

Alexandra J. Wright[1,2], Hans de Kroon[3], Eric J. W. Visser[3], Tina Buchmann[4], Anne Ebeling[5], Nico Eisenhauer[1,6], Christine Fischer[7,8], Anke Hildebrandt[5,7,9], Janneke Ravenek[3], Christiane Roscher[5,10], Alexandra Weigelt[1,6], Wolfgang Weisser[11], Laurentius A. C. J. Voesenek[12] and Liesje Mommer[13]

[1]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103 Leipzig, Germany; [2]FIT – Science & Mathematics, 227 W 27th St., New York, NY 11201, USA; [3]Experimental Plant Ecology, Institute for Water and Wetland Research, Radboud University, 6500 GL Nijmegen, the Netherlands; [4]UFZ, Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstrasse 15, 04318 Leipzig, Germany; [5]Institute of Ecology, Friedrich Schiller University Jena, Dornburger Straße 159, 07743 Jena, Germany; [6]Institute of Biology, Leipzig University, Johannisallee 21, 04103 Leipzig, Germany; [7]Institute of Geoscience, Friedrich-Schiller-University Jena, Burgweg 11, D-07749 Jena, Germany; [8]Department of Conservation Biology, UFZ-Helmholtz Centre for Environmental Research, Permoserstrasse 15,  04318 Leipzig, Germany; [9]Max Planck Institute for Biogeochemistry, Hans-Knöll-Straße 10, 07745 Jena, Germany; [10]Physiological Diversity, UFZ-Helmholtz Centre for Environmental Research, 04318 Leipzig, Germany; [11]Lehrstuhl für Terrestrische Ökologie, Technische Universität München, 85354 Freising, Germany; [12]Plant Ecophysiology, Institute of Environmental Biology, Padualaan 8, 3584 CH, Utrecht, the Netherlands; [13]Plant Ecology and Nature Conservation Group, Wageningen University, PO Box 47, 6700 AA Wageningen, the Netherlands

## Summary

- Flooding is expected to increase in frequency and severity in the future. The ecological consequences of flooding are the combined result of species-specific plant traits and ecological context. However, the majority of past flooding research has focused on individual model species under highly controlled conditions.
- An early summer flooding event in a grassland biodiversity experiment in Jena, Germany, provided the opportunity to assess flooding responses of 60 grassland species in monocultures and 16-species mixtures. We examined plant biomass, species-specific traits (plant height, specific leaf area (SLA), root aerenchyma, starch content) and soil porosity.
- We found that, on average, plant species were less negatively affected by the flood when grown in higher-diversity plots in July 2013. By September 2013, grasses were unaffected by the flood regardless of plant diversity, and legumes were severely negatively affected regardless of plant diversity. Plants with greater SLA and more root aerenchyma performed better in September. Soil porosity was higher in higher-diversity plots and had a positive effect on plant performance.
- As floods become more frequent and severe in the future, growing flood-sensitive plants in higher-diversity communities and in soil with greater soil aeration may attenuate the most negative effects of flooding.

## Introduction

Climate models suggest that flooding events may increase in occurrence and severity in the near future (Hirabayashi *et al.*, 2013; Jongman *et al.*, 2014). Large-scale periodic flooding events often have negative effects on economically important crops (Morris & Brewin, 2014), though floods may have variable effects on natural habitats depending on ecosystem type, disturbance regime, and flood severity (Odum *et al.*, 1979; Silvertown *et al.*, 1999; Vervuren *et al.*, 2003; Voesenek *et al.*, 2004; Wright *et al.*, 2015b; Fischer *et al.*, 2016). In fact, the effects of large-scale flooding events on plant communities are probably the combined result of species-specific flooding tolerance (Van Eck *et al.*, 2004; Mommer *et al.*, 2006), flood and biodiversity-induced changes in resource availability (Odum *et al.*, 1979; Wright *et al.*, 2015b), and altered ecological interactions that can

either exacerbate or ameliorate the negative effects of floods (Toogood *et al.*, 2007; Violle *et al.*, 2010).

The negative effects of flooding on individual plant performance result mainly from restricted gas exchange owing to slow gas diffusion in water compared with air, as well as low light intensities in turbid flood waters (Visser *et al.*, 2000; Vervuren *et al.*, 2003; Bailey-Serres & Voesenek, 2008). This leads to an energy and carbohydrate crisis in plant tissues (Mielke *et al.*, 2003; Sasidharan & Voesenek, 2015), which reduces plant growth and, ultimately, survival (Mommer *et al.*, 2006). Plant species differ in their ability to survive the adverse effects of flooding as a result of both constitutive and induced differences in plant traits, such as plant height, aerenchyma production, adventitious roots, modifications of the leaf anatomy, enhanced shoot elongation, hyponasty, barriers against radial oxygen loss, and starch storage (Bailey-Serres & Voesenek, 2008; Voesenek &

Bailey-Serres, 2015). In particular, taller plants with higher specific leaf area (SLA) and greater aerenchyma content can maintain higher amounts of gas exchange during a flood and therefore continue growing (Mommer & Visser, 2005; Colmer & Voesenek, 2009). Further, some plants may store large amounts of starch in their below-ground structures, modify their metabolic rates, and have the capacity to grow rapidly after the flood waters recede (Voesenek & Bailey-Serres, 2015).

Most of the previous work on species-specific flooding tolerance focused on particular model species (*Arabidopsis thaliana*, *Rumex* spp., and *Oryza* spp.), growing relatively young plants in small pots under highly controlled conditions (Colmer & Pedersen, 2008; Chen *et al.*, 2009; Vashisht *et al.*, 2010). However, when flooding occurs in natural communities, many additional factors may come into play that fundamentally alter plant performance over expectations from plants growing in isolation. Past work, in the field of community ecology, has shown that higher-diversity plant communities are often more resistant to environmental disturbances and may respond positively to some mild flooding events compared with plants growing in monoculture (Reich *et al.*, 2001; Isbell *et al.*, 2015; Wright *et al.*, 2015b). These effects may partially result from altered microsite conditions in plant mixtures, which can result in improved performance of individual plants (Tilman *et al.*, 1997; Allan *et al.*, 2013; Fischer *et al.*, 2015; Wright *et al.*, 2015a). For example, higher-diversity plant communities have increased rooting biomass (Mueller *et al.*, 2013; Ravenek *et al.*, 2014) and thus a higher potential for increasing total porosity below ground (Fischer *et al.*, 2015). Higher-diversity plant communities may buffer against the decrease in soil air porosity that accompanies a flooding event (Silvertown *et al.*, 1999). While the environment immediately surrounding a plant (the microsite) is theoretically important, no research to date has examined the role of species-specific traits and the ecological context of the plant community in determining plant responses to flooding (but see Brotherton & Joyce, 2015 for a conceptual review).

Here we report on a natural flooding event that occurred in June 2013 (early summer in Europe) in the Jena Experiment, a long-term biodiversity experiment in central Germany (Roscher *et al.*, 2004). We quantified natural variation in several morphological and physiological traits (plant height, SLA, root aerenchyma, below-ground starch) in 60 plant species (reduced to 40 with all traits available), clustered in four functional groups (grasses, legumes, small herbs, and tall herbs). We assessed how plant traits and microsite variables (soil air porosity of the plot) affected species-specific biomass production in monocultures and 16-species mixtures at two different points in time following the subsidence of the flood. We tested the following two hypotheses:

(1) Species performance in monoculture after flooding is dependent on morphological and physiological traits related to flooding tolerance (plant height, SLA, aerenchyma, starch reserves) and related plant functional groups (e.g. most grasses are tall). The former three traits are expected to determine plant performance during short-term submergence. Conversely, starch reserves are probably related to anaerobic metabolism and post-flood recovery in the longer term.

(2) Species-specific responses to flooding are altered when plants are grown in higher-diversity mixtures. Species-specific responses in mixture are more affected by ameliorated environmental conditions in higher-diversity plots (e.g. air porosity of soils) than by physiological traits or related plant functional groups.

## Materials and Methods

The Jena Experiment is located in central Germany on the banks of the Saale River (http://www.the-jena-experiment.de/skin/jenaexperiment/images/JenaExperiment_jpg). The experiment mimics a mesophilic grassland and is situated on Eutric Fluvisol soils (Roscher *et al.*, 2004). The experiment was established in 2002 using 60 herbaceous species (16 grasses, 12 legumes, 12 small herbs, and 20 tall herbs) that were sown on bare ground after tilling and treatment with glyphosate in summer 2001 to reduce further weed establishment (see Roscher *et al.*, 2004 for detailed methods and species lists). The plots have since been weeded several times $yr^{-1}$ to remove nontarget species (not in the original 60 species set). Plots are not fertilized. Plots are mown and mown plant material is removed twice per year to simulate management of extensively used hay meadows in the region (mown to 5 cm using cutter bars).

The main experiment of the Jena Experiment consists of 82 grassland field plots of $20 \times 20$ m representing a gradient in species richness (1, 2, 4, 8, 16 and 60 species). Monocultures of all 60 species were established on neighboring $3.5 \times 3.5$ m plots in 2002 (reduced to $1 \times 1$ m in 2010). In addition, new monocultures of all species in 1 $m^2$ plots were established in 2010 – these plant communities are younger and were used for the determination of plant traits (plant SLA and height), but not for the assessment of community responses to flooding as a result of expected changes in plant communities over time (Reich *et al.*, 2012). Combining monoculture plots in the main experiment with the supplemental monoculture plots established at the same time resulted in a total of 85 monocultures that were assessed in the current study (with some, but not all, of the 60 species replicated in monoculture). Plots were distributed in four blocks parallel to the riverside with an even number of plots per species-richness level in each block. In the current analysis, we compare monocultures with those same species in high-diversity mixtures. We use the 16-species mixtures as the high-diversity comparison (instead of 60-species mixtures) because of the low replication of the 60-species plots (four replicates and no replication at different flooding intensities), while the 16-species mixtures exist with 14 replicates and three or four replicates per block. Therefore, the 16-species mixtures were more suitable for assessing the flooding intensity gradient and the mixture effects simultaneously.

### June 2013 flood and site conditions

Rainfall at the Jena site in May 2013 was *c.* 150 mm and 25% of the total rainfall in 2013. This was nearly three times greater than the long-term average for the site (Supporting Information Table S1). The daily average air temperature during the flood year was within the range of longer-term averages, although

slightly cooler than average in June 2013 (Table S1). On 30 May 2013, water overtopped the banks of the Saale River and inundated the plots at the site. Plots experienced between 4 and 24 d of continuous flooding. The water height reached up to 60 cm above the soil surface, though this varied depending on slight variations in topography at the field site (Fig. S1).

For the purposes of comparison across plots, we quantified a flooding index (FI) for each plot by recording the number of days of flooding that each plot experienced and the percentage cover of flood waters (0%, 25%, 50%, 75%, 100%) on each of those days (FI $= \sum_{day=1}^{day=24} \mathrm{RatioFlooded_{day}}$, *sensu* Wright *et al.*, 2015b). The FI was also highly correlated with the maximum depth of the water recorded (though the field site was impassible on the day of actual high water and water was probably up to 20 cm deeper than what was recorded; Fig. S1).

### Biomass sampling and flood responses

Above-ground biomass was sampled in all plots between 29 and 30 May 2012, 31 August and 1 September 2012, 7 and 8 July 2013 (2 wk after the flood waters receded), and 19 and 20 September 2013 by randomly placing two $0.2 \times 0.5$ m rectangles in the large plots. Then, biomass was harvested at 3 cm above the soil surface, separated to the species level, dried at 70°C (48 h), and subsequently weighed. Samples harvested after the flood in July 2013 were carefully flushed with water to remove any dirt from the flood. For all analyses (both monocultures and mixtures) we compared species-specific biomass per plot over time.

To assess species-specific responses to flooding and to account for natural seasonal variation in plant growth at the site, we calculated a log-relative response ratio for each species in each plot in comparison to that species and plot at a similar point during the previous year. For the July 2013 sampling date, the harvest occurred approximately midway between the late May (37 d) and the late August (55 d) sampling dates of the previous year. We therefore calculated a July 2013 log response ratio by comparing with the average of the previous year:

$$\mathrm{JulyLOGRR} = \mathrm{Log_e}\left(\frac{\mathrm{July\ 2013}}{\mathrm{Ave(May\ 2012, August\ 2012)}}\right)$$

The September 2013 harvest was only 20 d from the previous year sample and thus we calculated the September 2013 log response ratio as a direct comparison with August 2012:

$$\mathrm{SeptemberLOGRR} = \mathrm{Log_e}\left(\frac{\mathrm{September\ 2013}}{\mathrm{August\ 2012}}\right)$$

Based on these calculations, we would expect large values ($> 0$) of July log response ratios to reflect plants that were capable of growing more, early in the season (including the period of the flood) than during the previous year. These plants responded more positively to flooding. We would expect similar values of September log response ratios to reflect plants that continued to grow well in the months following the flood or that re-grew rapidly after the floodwater receded. For both indices, a value of 0 reflects no change in comparison with the previous year, and a value of $< 0$ reflects a decline in growth relative to the previous year.

Importantly, the field site was also mown at different times during the 2 yr (June 2012, September 2012, July 2013 and September 2013; Fig. S2). Consequently, for all log response ratios we are conservative in our interpretation of values near 0 and focus more on relative comparisons between groups. Furthermore, to assess whether 2013 trends were anomalous compared with longer-term interannual variability, we also compared 2013 harvest data with the averages from 2010, 2011 and 2012 using log response ratios. These results are very similar and confirm the results of the 2012 vs 2013 contrast (Figs S3, S4).

### Plant height and maximum SLA

On 13 June 2013 we assessed species-specific shoot height (at least three specimens per species) of all 60 species growing in the $1 \times 1$ m monoculture plots that were established in 2010. We chose these monoculture plots for the sampling because they are all within a single block and experienced a similar degree of flooding. We sampled height and SLA for four species in the older monoculture plots (established in 2002) because they were not available in the later established monocultures (*Pastinaca sativa*, *Primula veris*, *Sanguisorba officinalis*, *Trifolium fragiferum*). Afterwards, the measured shoots were cut at the ground level in the field and stored in plastic bags in a cooler to prevent drying. In the laboratory, all shoots were carefully cleaned of dirt. Then we measured the stretched shoot length (cm) of stems. SLA was assessed by selecting three to five fully expanded leaves per shoot per species. Leaf area was determined using a leaf area meter (LI-3100 Area Meter; Li-Cor, Lincoln, NE, USA). Leaves were then dried for 48 h at 70°C and weighed. Shoot length and SLA were averaged per monoculture to get mean and maximum values per species.

### Assessment of aerenchyma abundance

On 22 June 2013, we harvested root biomass of 42 of the 60 species from the most severely flooded area at the field site (we could not find samples of the other 18 species; Table S2). Thus, root biomass does not correspond to particular plots but instead corresponds to several areas of the field near the 60-species plots that had been flooded for *c.* 21 d. These locations were chosen in order to avoid destructive root sampling within the main experiment. Root samples were harvested from plants that had sufficient surviving shoot biomass to identify species. Root biomass near the base of the plant was harvested and stored together with a small piece of connected shoot biomass. Fresh root samples were cut, stored in 70% (v/v) ethanol, then infiltrated in tap water, and sliced by hand using a razor blade. These sections were then inspected and photographed with a Leica DM2500 microscope and DFC420C microscope camera (Leica Microsystems GmbH, Wetzlar, Germany). We sampled three individuals of each species from the field site. The vast majority of these species were nonwetland species and thus had lower aerenchyma content than typical wetland model species (e.g. *Rumex*). Unfortunately,

when larger-scale aerenchymatous tissue is not present, it is difficult to make cell-to-cell assignments of whether cells are alive, dead, gas-filled, or water-filled. For this reason, we used a coarse-ranked categorization of aerenchyma content, as opposed to a precise but potentially inaccurate quantification of porosity using image analysis. We quantified aerenchyma content of root samples using a five-point ranking system (Fig. S5, assessed according to Visser & Bogemann (2003)). All rankings were assigned and verified using a second sample of the same species from the field site. Samples with no visible aerenchyma were recorded as zero, samples with one to two lysed cells were recorded as one, samples with ~ 30–50% of aerenchyma in a single layer of cortex were recorded as two, samples with 50–90% of aerenchyma were recorded as three, and samples with a continuous ring of aerenchyma were recorded as four (Fig. S5).

## Starch content

During the aerenchyma harvest, we also harvested root biomass of the same plants (where available) for starch assays for 40 of the 60 species in the Jena Experiment (Table S2). We sampled roots from two to three individuals in the field, placed these samples immediately in liquid nitrogen, and stored these at $-80°C$ for laboratory analyses. Freeze-dried, ground root tissue was aliquoted in samples of ~ 20 mg, suspended in 500 µl of 0.83 M perchloric acid, vortexed, and then centrifuged for 15 min at 4°C and 17 950 $g$. Pellets were washed twice in 80% ethanol, air-dried, and used for starch determination using a commercial starch determination kit (K-TSTA; Megazyme International, Wicklow, Ireland), which provided the chemicals for the enzyme reactions. At 100°C, 250 µl thermostable α-amylase solution hydrolyzes the starch in the pellets into soluble branched and unbranched maltodextrins. After that, 10 µl amyloglucosidase solution was used to quantitatively hydrolyze maltodextrins to D-glucose, resulting in a total volume of 260 µl reaction sample. Subsequently, 250 µl glucose oxidase/peroxidase solution was added to 10 µl of reaction sample, in which D-glucose is oxidized to D-gluconate with the release of hydrogen peroxide, followed by a colorimetric reaction employing peroxidase and the production of aquinoneimine dye. Samples were measured spectrophotometrically in a BioTek plate reader at 510 nm.

## Soil air porosity

Volumetric soil water content and total soil porosity of each of the plots in the main experiment were determined on 24 June 2013 immediately after the floodwater had receded. Both measurements were determined using one soil core sampled per plot (57 mm diameter × 40.5 mm height, $V_s$ = inner volume of 100 $cm^3$). Samples were weighed within 30 min of sampling ($m_f$). Samples were then placed on a sand bed box with water level held constant to allow for saturation ($m_w$). After 48 h the samples were weighed wet, oven-dried at 105°C, and weighed again to determine DW ($m_d$). We then calculated total soil porosity (%) using the density of water ($p_w = 1 \, g \, cm^{-3}$) ($n = 100 \times (m_w - m_d)/(p_w \times V_s)$) and volumetric soil water content (%) as $\theta = 100 \times (m_f - m_d)/(p_w \times V_s)$. Finally, soil air porosity was calculated as soil porosity minus volumetric soil water content per plot (Blume et al., 2011).

## Statistical analyses – monocultures

We classified species monocultures according to one of four functional groups and then assessed functional group responses to flooding in July and September 2013. We used a mixed-effects model that included fixed effects for FI and functional group and a random effect for species identity. Owing to the lack of replication of monocultures (and variable flooding intensity among monocultures), we could not assess species-specific responses to flooding (instead we assessed average monoculture responses per functional group). We ran these functional group analyses using both the July log response ratio and the September log response ratio as response variables. We ran all analyses using JMP Pro v.11 (SAS Institute Inc., Cary, NC, USA).

We then ran a second mixed-effects ANCOVA (Gotelli & Ellison, 2012) to assess the role of associated morphological and physiological traits: in particular, how aerenchyma content (ordinal), plant height (continuous), SLA (continuous), and starch content (continuous) contribute to functional group flooding responses. For this analysis, our results were confined to the 40 species for which we had a complete dataset (Table S1). We included fixed effects for functional group and FI (as earlier) plus trait data for plant height, maximum SLA, aerenchyma content, and starch content. We also included an interaction term for starch content and functional group because starch content varied by two orders of magnitude between grasses and the other three functional groups. Because of the low sample size, we could not include interactions for all fixed effects with functional group.

To assess trait clustering and potential combinations of traits that may explain species responses in monoculture, we also conducted a principle component analysis (PCA) using all measured traits, and species-specific responses in July 2013 and September 2013 separately.

## Statistical analyses – mixtures

To assess how species-specific responses (lumped by functional group) varied in higher-diversity plant communities (our second hypothesis) we compared species-specific log response ratios (July and September) in monocultures vs 16-species mixtures. We ran a set of mixed-effects models that assessed fixed effects for FI, functional group, diversity of the plot (monoculture or mixture), and an interaction between functional group and diversity of the plot. We included random effects for species identity and plot identity nested within block to account for spatial autocorrelation of multiple measurements taken within the same plot and the spatial blocking factor at the site (Roscher et al., 2004).

## Statistical analyses – community and trait-based mechanisms

We also assessed how soil air porosity changed in response to flooding intensity and how this was affected by community

diversity using a two-way ANOVA with flooding intensity, diversity, and first-order interactions assessed. To explore the mechanisms that explain species-specific responses to flooding in mixture vs monoculture (our second hypothesis) we ran a stepwise regression model including all species traits and community microsite variables (FI, soil porosity, community diversity, species functional group, species height, SLA, aerenchyma content, starch content) and all first-order interactions on July 2013 species-specific log response ratios and September 2013 species-specific log response ratios. We selected the best-fit model using minimum Bayesian inference criteria (BIC) and forward selection criteria. The most significant effects were sequentially added to the model until all significant effects were added.

We then built separate mixed-effects models for July 2013 and September 2013 that always included fixed effects for community diversity (experimentally manipulated and therefore included regardless of stepwise regression results) plus only those factors selected by the stepwise regression. In these secondary models we included random effects for species identity and plot nested

within block to account for spatial autocorrelation of multiple measurements taken within the same plot and the spatial blocking factor at the site.

## Results

### Species performance in monoculture

In July 2013, monocultures suffered strongly in severely flooded plots. In particular, monocultures that experienced 15 d or more of flooding had as little as 1% of the biomass in July 2013 compared with the previous year, while monocultures that experienced less than 4 d of flooding grew similarly or even more in July 2013 than in the previous year (FI effect: $F_{1,69} = 12.0$, $P < 0.001$, Fig. 1a). Across the flooding gradient, grasses were less negatively affected by the flood in July than any of the other functional groups (functional group effect, $F_{3,34} = 5.8$, $P = 0.0003$; no FI × functional group interaction, Fig. 1b).
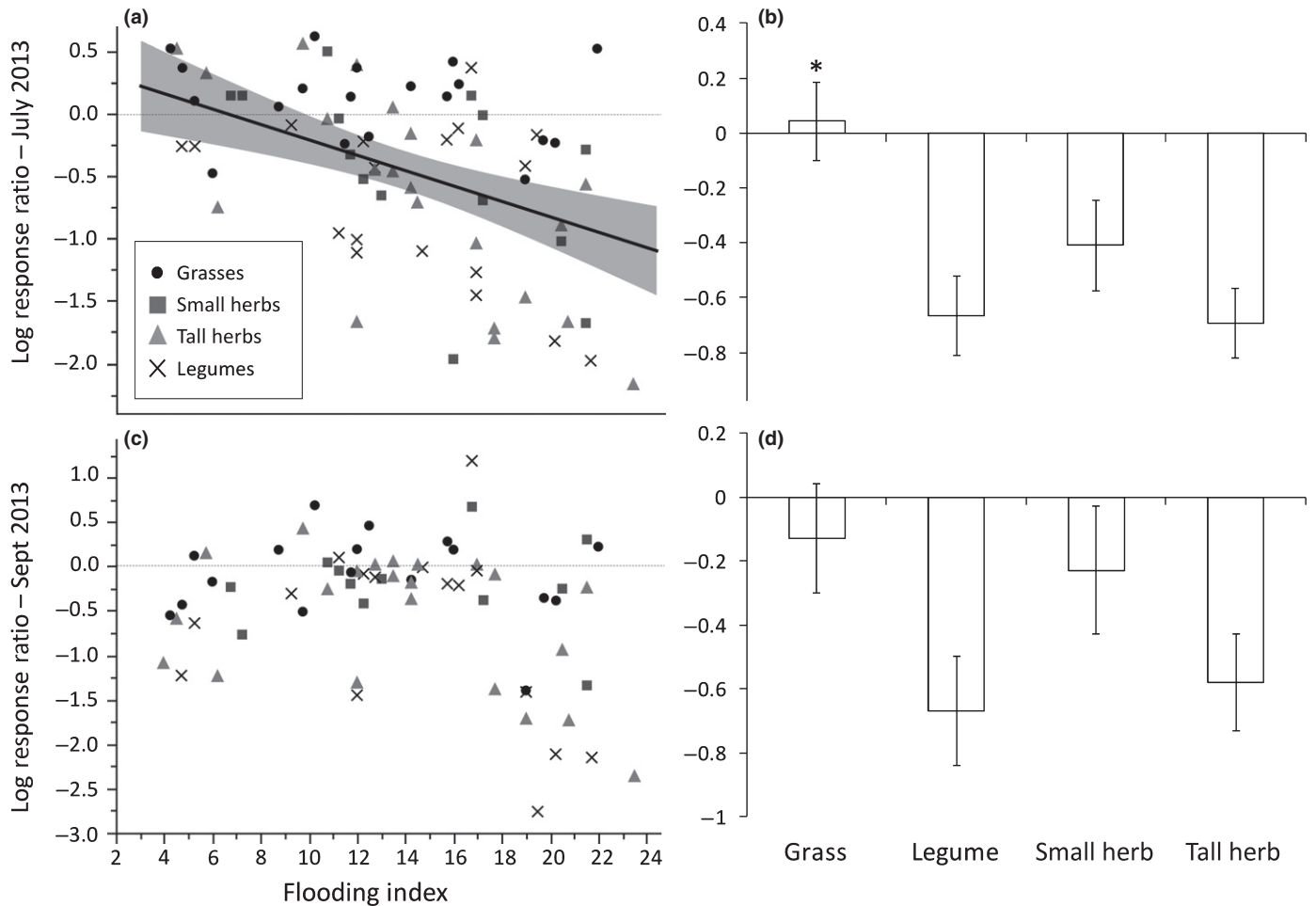


Fig. 1 Species-specific plant responses to flooding in monocultures. We compared species-specific biomass production in the year of the flood (2013) with biomass production at similar points during the season in 2012. We measured plant recovery at two points in time: immediately following the flood (July 2013; a, b) and 2 months after the flood ended (September 2013; c, d). We assessed species-specific responses to flooding intensity (a, c) and average functional group responses to the flood (b, d). Values greater than zero reflect growth improvements over the previous year. Values less than zero reflect growth declines. Shaded areas represent 95% confidence intervals (only shown for significant relationships) and error bars represent ± SE of the mean. A significant difference ($P < 0.05$) is indicated with an asterisk in (b).

When including specific plant traits in the monoculture analysis (plant height, SLA, aerenchyma content, starch content), we found that none of the individual traits significantly affected plant species performance in July 2013 (Table 1; Fig. 2a,b). However, the combination of these four traits in our monoculture analysis did explain the improved performance of the grasses (functional group was no longer significant; Table 1). We also ran a PCA with all plant functional traits in July (i.e. log response ratios) and found that improved performance of grasses in July 2013 was clustered most closely around plant height (and oppositely correlated with starch; Figs S6, S7). Furthermore, taller plants, such as *Arrhenatherum elatius*, were more likely to emerge above the highest watermark recorded (June 7); this was particularly important in the most severely flooded plots (Fig. S8).

In contrast to July responses, neither FI ($F_{1,64} = 2.0$, $P = 0.16$) nor functional group ($F_{3,28} = 2.1$, $P = 0.13$) significantly affected plant performance by September 2013 (Fig. 1c,d). Conversely, plant traits in September determined plant performance regardless of functional group. Plants with greater maximum SLA (Fig. 2c), greater aerenchyma content (Fig. 2d), and legumes with greater starch content (Fig. S9) performed better in September 2013 compared with the previous year.

## Species performance in monoculture vs mixture

In mixtures in July 2013, we observed similar species-specific patterns associated with functional group as those patterns observed in monocultures. Flooding had a negative overall effect on species-specific growth (Table 2), although grasses grew better than the other functional groups (Fig. 3). Importantly, while all plants (in mixture and monoculture) grew, on average, slightly less than in the previous year, plants in mixture were less strongly affected by this than plants in monoculture. In other words, species-specific flooding effects were buffered in 16-species mixtures (Fig. 3a).

By September 2013, some functional groups grew better in mixture than in monoculture, while others were unaffected

**Table 1** We measured the above-ground plant biomass of all monocultures in July 2013 and compared it with the average biomass of monocultures in the previous year (log response ratio)

| Source | Response in July | | | Response in September | | |
|---|---|---|---|---|---|---|
| | df | *F*-ratio | *P*-value | df | *F*-ratio | *P*-value |
| Flood index | 1,32 | 16.9 | **0.0003*** | 1,35 | 2.68 | 0.11 |
| Functional group | 3,21 | 0.24 | 0.87 | 3,23 | 1.43 | 0.26 |
| Height | 1,14 | 0.09 | 0.76 | 1,17 | 3.07 | 0.10 |
| Max SLA | 1,20 | 0.85 | 0.37 | 1,19 | 4.87 | **0.04*** |
| Aerenchyma | 4,20 | 0.26 | 0.90 | 4,19 | 2.97 | **0.04*** |
| Starch content | 1,17 | 1.09 | 0.31 | 1,17 | 1.57 | 0.23 |
| Starch × functional group | 3,21 | 1.37 | 0.28 | 3,22 | 4.26 | **0.02*** |

We also measured biomass of monocultures in September 2013 and compared it with the average biomass of monocultures in August 2012. In all analyses, we included species identity as a random effect. Bold text and asterisks indicate significant effects ($P < 0.05$). SLA, specific leaf area.

(Fig. 3c,d). Specifically, the biomass of small and tall herbs was enhanced in mixtures (returning to near 2012 values) but these same functional groups had grown 30–60% less than their 2012 biomass when growing in monocultures (Fig. 3d). By contrast, legumes grew up to 60% less than the previous year and this was true whether growing in mixture or monoculture (Fig. 3d).

## Species performance in monoculture vs mixture: community and trait-based mechanisms

Soil air porosity was almost 15% lower in severely flooded plots compared with less severely flooded plots on 24 June 2013 (i.e. at the end of the flood (Fig. S10); $F_{1,23} = 8.4$, $P = 0.008$). Soil air porosity was almost 4% higher in diverse plots than in monoculture plots across the flooding severity gradient (Fig. S10; $F_{1,23} = 5.56$, $P = 0.03$). There was no difference in how flooding intensity affected soil air porosity in high- vs low-diversity plots (no flooding intensity × diversity interaction, $F_{1,23} = 0.53$, $P = 0.47$).

In our assessment of the mechanisms responsible for plant performance in mixture vs monoculture, we included all plant traits and soil porosity of the plot. In July 2013, the only factors that were significant in the stepwise regression (combining all species-specific effects in monoculture and mixture) were FI, functional group, plant height, and the interaction between FI and functional group (Table S3). Regardless of plant community diversity, species-specific responses in July across all plots (monocultures and mixtures) were most strongly related to flooding intensity (Table S4). Furthermore, grasses grew well regardless of flooding intensity, whereas everything else grew less when growing in more severely flooded plots (Fig. 4b). Plant height again played a minor role, as indicated by the selection of height in the stepwise regression (but nonsignificant in the full model that included random effects for plot identity). Soil air porosity had no significant effect on plant performance in July (Fig. 4a).

In September 2013, the only factors that were significant in the stepwise regression were soil air porosity, functional group, and an interaction between soil air porosity and functional group (Table S5). When compared across plot mixtures and monocultures, functional traits did not mediate species responses. Species-specific responses were most strongly related to differences among functional groups and functional group-specific responses to the air porosity of soils (Table S4). Higher-diversity plots had greater air porosity, and legumes, in particular, grew significantly less than in previous years when growing in low-air-porosity soils (Fig. 4c). All other functional groups had recovered by September 2013 (Fig. 4c).

## Discussion

In the short term (July 2013), plant species were less negatively affected by flooding when growing in species-rich plant communities than when growing alone in monoculture. In monocultures, plants produced an average of 40% less biomass compared with the previous year, whereas in mixture this drop in productivity was only 20%. By September 2013, the biodiversity effect
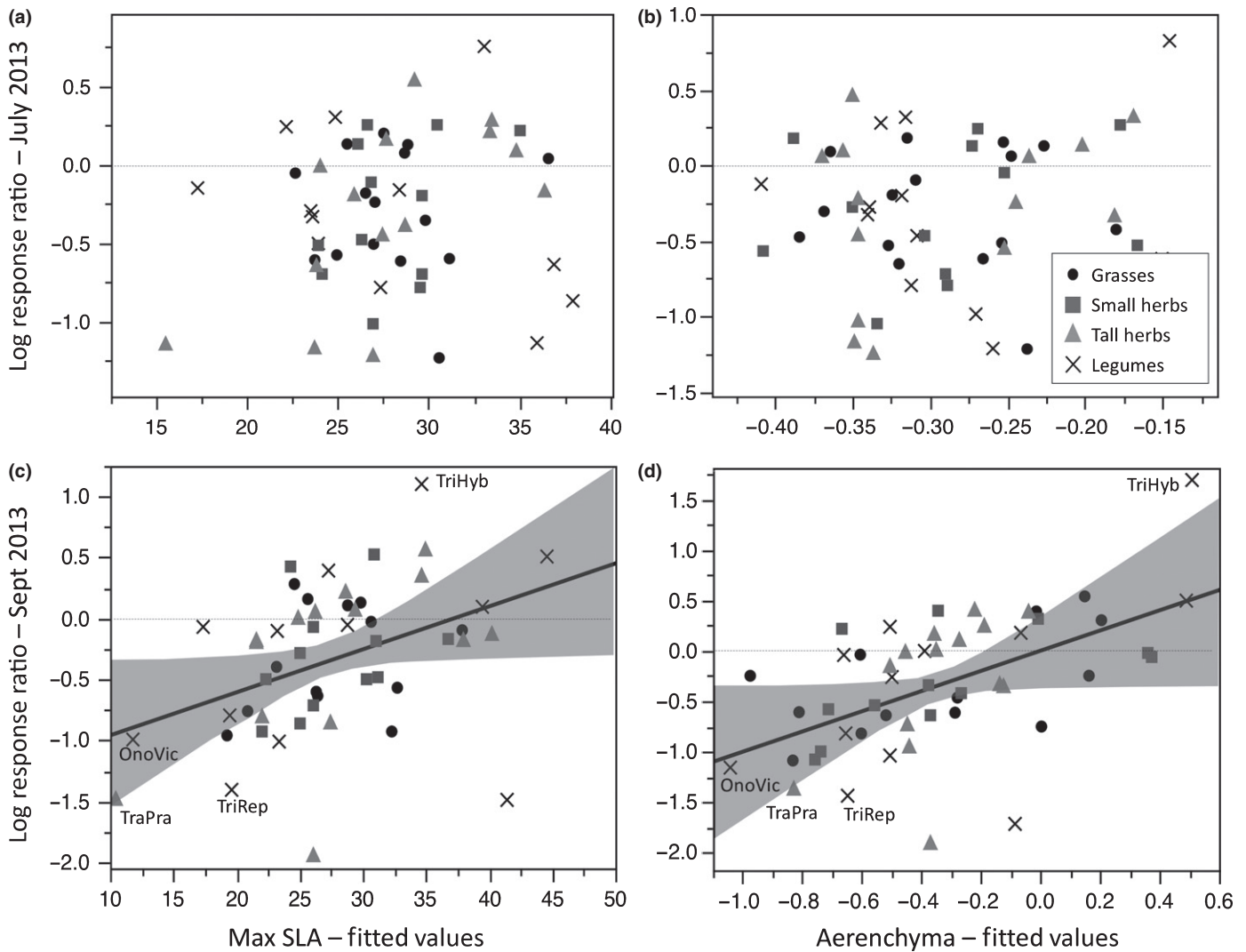
**Fig. 2** The effects of specific leaf area (SLA) and aerenchyma content on species-specific responses in monoculture in July 2013 (a, b) and September 2013 (c, d). Lines indicate significant relationships, shaded areas represent 95% confidence intervals. Species discussed in the text are labeled (TriHyb, *Trifolium hybridum*; OnoVic, *Onobrychis viciifolia*; TriRep, *Trifolium repens*; TraPra, *Tragopogon pratensis*).

**Table 2** We measured species-specific, above-ground plant biomass in mixtures and monocultures in July 2013 and September 2013

| Source | Response in July | | | Response in September | | |
|---|---|---|---|---|---|---|
| | df | F ratio | P-value | df | F ratio | P-value |
| Flood index | 1,35 | 30.6 | **< 0.0001*** | 1,38 | 4.21 | **0.05*** |
| Functional group | 3,57 | 7.61 | **0.0002*** | 3,57 | 4.12 | **0.01*** |
| Plot mixture | 1,57 | 4.56 | **0.04*** | 1,15 | 3.53 | 0.08 |
| Functional group × mixture | 3,173 | 1.04 | 0.38 | 3,143 | 3.11 | **0.03*** |

We compared the log response ratio at both sampling dates with the previous year. All analyses include the fixed effects of flooding index, functional group, mixture effects (monoculture or mixture), and interactions between functional group and mixture effects, as well as random effects for species identity, and plot nested within block to account for spatial autocorrelation according to a blocking factor. Bold values and asterisks indicate significant effects ($P < 0.05$).

largely depended on functional groups: small and tall herbs recovered to pre-flood levels, but only when growing in higher-diversity mixtures. The grasses grew similarly well in monoculture and mixture – in all cases grasses grew just as much as in the previous year. The biodiversity effect was also weak for legumes, which grew similarly poorly in mixture and monoculture communities. On average, legumes grew 60% less by September 2013 than in the previous year, indicating that legumes were strongly negatively affected by the flood, perhaps as a result of the deleterious effects of anoxic conditions on nodule activity (James & Crawford, 1998). This negative legume response was buffered when growing in soils with greater air porosity.

## Monoculture responses to flooding – July

For grasses, the average responses to flooding were neutral or positive, even after a relatively long flooding period (i.e. > 20 d in some plots). This may be because of the greater average height of
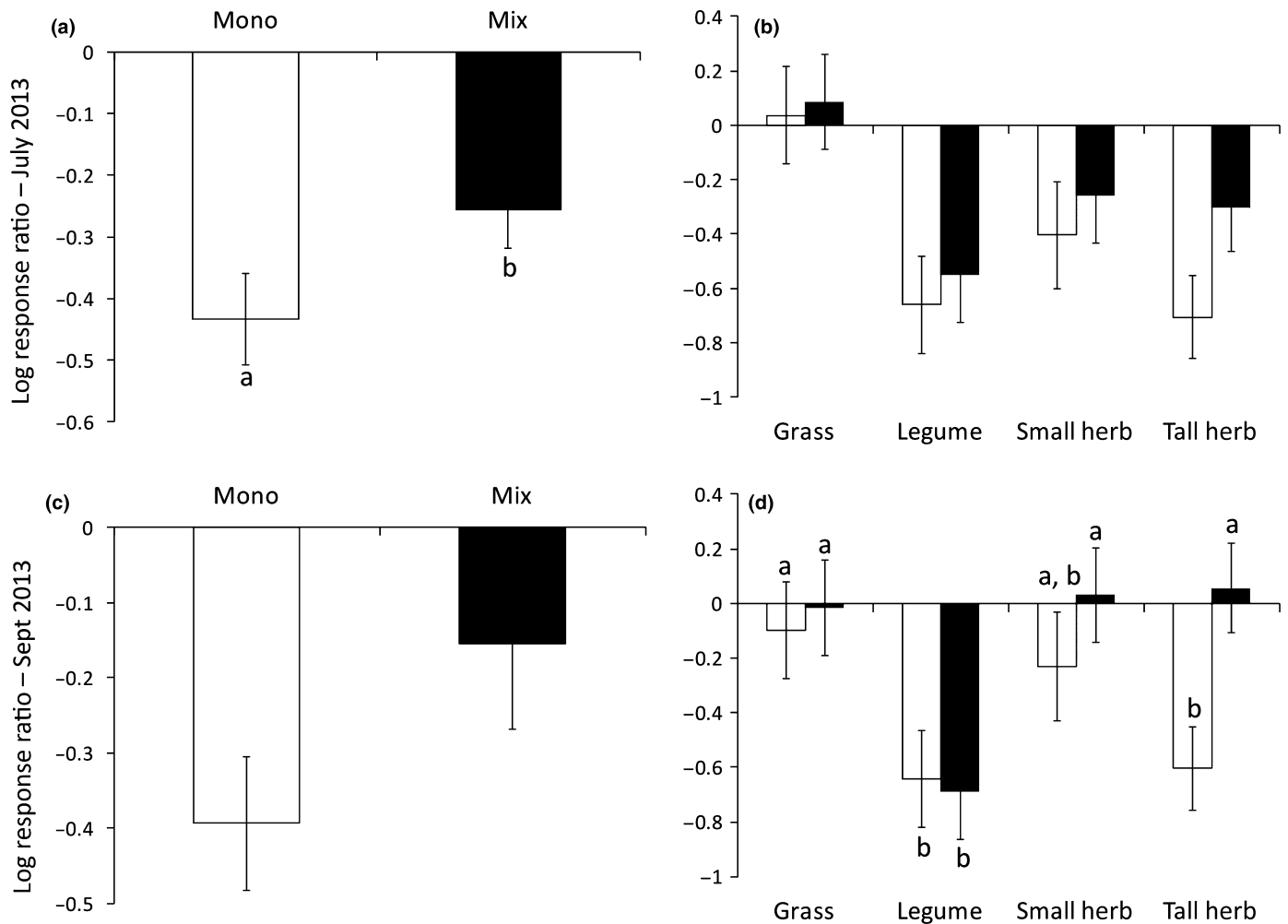
**Fig. 3** Average species-specific responses to flooding and functional group responses to flooding in July 2013 (a, b) and September 2013 (c, d). Species-specific responses in mixture are shaded black, while species-specific responses in monoculture are in white. Error bars indicate ± SE of the mean. Significant differences are indicated with letters (differences determined using *post hoc* Tukey tests). We have included all comparisons for both sampling dates for ease of comparison over time. However, some relationships (c) or interactions (b) are not significant (thus letters are not included).

grasses (clustering of plant height with positive July responses for grasses; Figs S6–S8). Indeed, the average height of species within the grasses was highest, at $34 \pm 2.8$ cm. Legumes, small herbs and tall herbs reached an average of $17 \pm 3.2$, $10 \pm 3.6$, and $21 \pm 2.8$ cm, respectively (Fig. S7). Plants with greater maximum height may be functionally superior during natural flooding events because taller plants have the capacity to maintain contact of leaves with the air above the floodwaters, allowing for aerial gas exchange (Voesenek *et al.*, 2004; Chen *et al.*, 2011). In fact, recent work suggests that plant height is the single most important filter for flooding survival in natural plant communities (Garssen *et al.*, 2015; Sasidharan & Voesenek, 2015).

## Monoculture responses to flooding – September

While immediate flooding tolerance was closely linked to average grass performance, longer-term responses (i.e. in September) were significantly related to particular plant traits (SLA and aerenchyma production) and did not cluster according to well-known functional groups. Past work has shown that plants with

greater SLA have a greater capacity for underwater gas exchange which positively affects plant survival (Mommer & Visser, 2005; Mommer *et al.*, 2005, 2006; Winkel *et al.*, 2016). Our work suggests that increased SLA may also positively affect recovery after a naturally occurring flood, potentially because SLA improves maintenance of plant functioning, which may not result in improved immediate growth (in July 2013), but may allow for a faster recovery (by September 2013). Past work suggests that plants that are more frequently flooded may indeed have greater SLA, on average, over time, as SLA may act as a filter in frequently flooded systems (Winkel *et al.*, 2016). We also demonstrate the importance of aerenchyma production for species-specific recovery following a flood. Aerenchyma in the root cortex can provide a conduit for gas exchange between above-ground tissues and below-ground structures (Visser *et al.*, 2000; Huber *et al.*, 2008). Similar to SLA, plants with more aerenchyma may maintain gas exchange and basic plant functioning throughout the flood, which may allow a faster recovery after the waters recede. In contrast to previous work in controlled conditions (both glasshouse and field experiments) using *Oryza* sp.
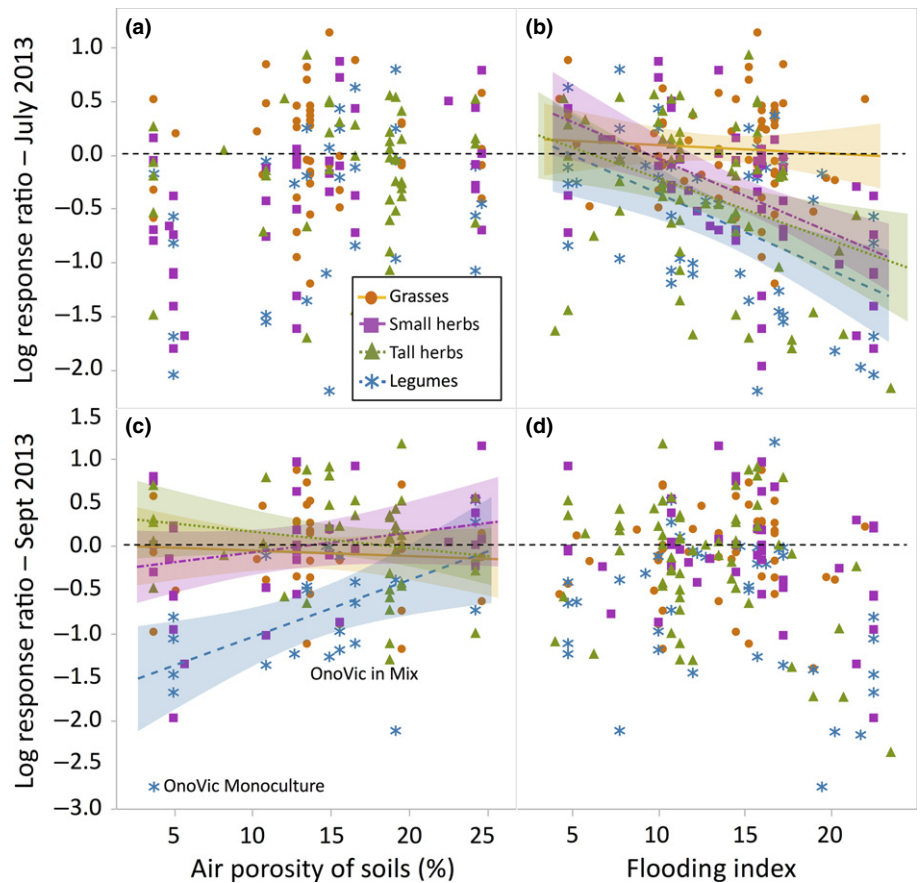
**Fig. 4** The effects of soil air porosity (%; a, c) and flooding index (b, d) on species-specific responses in July (a, b) and September (c, d). We measured air porosity of soils (%) and flooding intensity in all plots of the main experiment on 24 June 2013. Lines indicate a significant relationship or significant interaction. Shaded areas represent 95% confidence intervals. Species mentioned in the text are labeled (OnoVic, *Onobrychis viciifolia*).

(Voesenek & Bailey-Serres, 2015), starch content was not a strong driver of improved performance of our perennial plants in a long-term grassland experiment. The only plant functional group whose response was related to starch content was that of legumes in September 2013. Legumes with more starch in their roots recovered more rapidly by September 2013, but this response was driven solely by the reduced growth of a single species (*Onobrychis viciifolia*) that had particularly low starch content and is a well-known dryland species (Hayot Carbonero *et al.*, 2011).

We demonstrate here that plants with higher SLA and aerenchyma production recovered best by September 2013, and these strategies did not cluster according to well-known functional groups (i.e. grasses, herbs, and legumes). At one end of the spectrum, *Trifolium hybridum* (legume) was the highest performing species in terms of SLA, aerenchyma production, and September performance. Past work has demonstrated that *T. hybridum* is indeed moderately flood-tolerant, particularly in comparison with other legumes (Heinrichs, 1970). Here the *T. hybridum* monoculture experienced almost 17 d of flooding, was probably submerged for some of that time (Table S2; Fig. S8), and grew twice as much as in previous years. Our data suggest that this may have been partially a result of its ability to maintain gas exchange across the large surface area of its submerged leaves and utilize aerenchyma for gas diffusion below ground (reviewed in Mommer & Visser, 2005). This improved physiological performance during the flood may have allowed it

to grow quickly in the months following the flood. Conversely, *Tragopogon pratensis* (tall herb) and *Trifolium repens* (legume) were two of the poorest performing species in terms of SLA, aerenchyma production, and September performance (Table S2). This is consistent with their habitat preference (Ellenberg values of 4–5 on a scale of 1–10 with 10 indicating aquatic plants (Ellenberg, 1979) and inability to germinate, survive, or grow in even a small amount of standing water (Fraaije *et al.*, 2015).

### Plant responses to flooding in mixture plant communities

While monoculture responses to natural flooding events give us some insight into species-specific tolerances, we found that, on average, plant species richness protected species from immediate flooding damage and buffered many of the most sensitive species from longer-term negative effects. In fact, soil porosity (a whole-plot plant community measurement) was a better predictor of individual plant performance in mixture than the plant traits examined in the above analysis. This may explain why moderate natural flooding events sometimes have positive effects on productivity of plant communities – but only when plant communities are grown in higher-diversity mixtures (Wright *et al.*, 2015b). We caution that our trait measurements in the present study were not taken across the diversity gradient: some measurements were taken in monoculture plots (height and SLA), while others were measured outside of the plots or near the 60-species

plots to avoid destructive below-ground harvests (aerenchyma and starch content). A single value for each morphological and physiological trait was assigned for each species. Future work should also explore the role of trait plasticity along the diversity gradient (Gubsch *et al.*, 2011), and how this may also contribute to flooding tolerance and community resilience.

Immediately after the flood (i.e. July 2013), we found that most species responded less negatively to the flood when growing in the higher-diversity plant communities. This July 'biodiversity' response may be related to increased microbial activity and nitrogen content of soils in higher-diversity plots immediately following the flood (Wright *et al.*, 2015b). Further, previous work showed that higher-diversity plant communities can have up to 500% increased carbon accumulation and up to 600% increased nitrogen accumulation than lower-diversity experimental plots (Fornara & Tilman, 2008; Cong *et al.*, 2014; Lange *et al.*, 2015). Higher-diversity plant communities can also increase the size and stability of soil aggregates (Pérès *et al.*, 2013), decrease soil bulk density, and increase soil porosity (Fischer *et al.*, 2015). These changes in nutrient availability and the physical structure of soils probably lead to altered below-ground conditions that affect species-specific plant performance during and after a flooding event.

By September 2013, these mitigating biodiversity effects on flooding responses varied depending on plant functional group. Grasses grew well regardless of flooding severity or diversity, but small herbs (> 15%) and tall herbs (> 25%) were strongly buffered by growing in higher-diversity plant communities. Legumes, however, were not buffered by growing in mixtures. In fact, legumes grew less than 60% of their biomass in September 2013 compared with the previous year – regardless of whether they were growing in monoculture or mixture. During this recovery stage, we found that the functional group-specific responses were largely a result of differences in soil air porosity among plots. These differences in soil air porosity may be related to the emergent effects of plant diversity on soils. The lower bulk density induced by higher soil organic carbon in diverse plots (Fischer *et al.*, 2015) may be related to higher macroporosity (Kay, 1996) and consequent changes in how flooding affects soil aeration (Votrubová *et al.*, 2003; Sněhota *et al.*, 2010). Specifically, entrapped air in the larger pores of the diverse plots may have prolonged the time before reaching an anaerobic state.

Importantly, when soils are aerobic, the symbiosis with $N_2$-fixing rhizobia gives legumes a competitive advantage over other functional groups competing for below-ground nitrogen reserves in these grasslands (Mulder *et al.*, 2002). However, the legume–rhizobia symbiosis is highly dependent on the availability of oxygen, because bacterial respiration during N fixation is mostly aerobic (James & Crawford, 1998). The anoxic conditions experienced during floods may have reduced the development and increased senescence of the nodules (Mylona *et al.*, 1995) explaining the negative effects of flooding on legume performance (Heinrichs, 1970). Legumes in our experiment were strongly negatively affected by the flood, and not directly affected by diversity. However, legume performance was strongly improved in plots with greater soil air porosity.

## Concluding remarks

Flooding events are generally negative for nonflood-adapted species (Van Eck *et al.*, 2004; Mommer *et al.*, 2006). In fact, previous work indicated that species-specific functional traits should predict their flooding responses (Colmer & Voesenek, 2009). Accordingly, we demonstrate here that, in general, grasses are more resistant to flooding than other herbaceous nonflood plain species (potentially related to average grass height) and that legumes are very sensitive to flooding stress. Further, the ability to form aerenchymatous tissue and leaves with a relatively high SLA (across functional groups) appear to be traits that improve species tolerance during a natural flooding event, but particularly during the recovery period. This may be an important insight into designing flood-resistant grasslands and crops for those areas where floods are expected to increase in the future.

We also demonstrate here that individual species respond less negatively to flooding when growing in higher-diversity plant communities (multispecies mixtures). This suggests that an understanding of plant traits is not enough to explain species distributions and abundances in flood-prone environments. This additional community effect may be a result of altered soil structure or potentially of cascading competitive and facilitative interactions between species in higher-diversity mixtures. Further work should focus on the particular mechanisms that confer such strong buffering effects, particularly for flood-sensitive species.

Increased world-wide occurrence and magnitude of flooding in the future (Stocker *et al.*, 2013; Voesenek & Bailey-Serres, 2015) may necessitate new solutions to the adverse effects of flooding on plant performance. Our work provides a new perspective related to plant species diversity and its effect on soil porosity that has the potential to mitigate the negative effects of flooding on plant performance in the future. It may explain why flooding events often have negative effects on economically important flood-sensitive crops grown in monocultures (Morris & Brewin, 2014), although floods may have variable effects on species growing in diverse natural habitats (Odum *et al.*, 1979; Deiller *et al.*, 2001; Vervuren *et al.*, 2003; Wright *et al.*, 2015b).

## Author contributions

A.J.W. measured aerenchyma, analyzed the data, and wrote the manuscript, H.d.K., N.E., C.R., A.W., A.E., W.W. and L.M. conceived the study and designed the experiment, E.J.W.V., T.B., A.E., C.F., A.H., J.R., C.R., L.A.C.J.V. and L.M. collected the data, and all authors contributed to data analysis and the final version of the manuscript.

## References

Allan E, Weisser WW, Fischer M, Schulze E-D, Weigelt A, Roscher C, Baade J, Barnard RL, Bessler H, Buchmann N *et al.* 2013. A comparison of the strength of biodiversity effects across multiple functions. *Oecologia* 173: 223–237.

Bailey-Serres J, Voesenek LACJ. 2008. Flooding stress: acclimations and genetic diversity. *Annual Review of Plant Biology* 59: 313–339.

Blume HP, Stahr K, Leinweber P. 2011. *Bodenkundliches Praktikum: Eine Einführung in pedologisches Arbeiten für Ökologen, Land- und Forstwirte, Geo- und Umweltwissenschaftler.* Heidelberg, Germany: Springer.

Brotherton SJ, Joyce CB. 2015. Extreme climate events and wet grasslands: plant traits for ecological resilience. *Hydrobiologia* 750: 229–243.

Chen X, Huber H, De Kroon H, Peeters AJM, Poorter H, Voesenek LACJ, Visser EJW. 2009. Intraspecific variation in the magnitude and pattern of flooding-induced shoot elongation in *Rumex palustris*. *Annals of Botany* 104: 1057–1067.

Chen X, Visser EJW, de Kroon H, Pierik R, Voesenek LACJ, Huber H. 2011. Fitness consequences of natural variation in flooding-induced shoot elongation in *Rumex palustris*. *New Phytologist* 190: 409–420.

Colmer TD, Pedersen O. 2008. Oxygen dynamics in submerged rice (*Oryza sativa*). *New Phytologist* 178: 326–334.

Colmer TD, Voesenek LACJ. 2009. Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology* 36: 665.

Cong W-F, Van Ruijven J, Mommer L, De Deyn GB, Berendse F, Hoffland E. 2014. Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *Journal of Ecology* 102: 1163–1170.

Deiller AF, Walter JMN, Trémolières M. 2001. Effects of flood interruption on species richness, diversity and floristic composition of woody regeneration in the Upper Rhine alluvial hardwood forest. *Regulated Rivers: Research & Management* 17: 393–405.

Ellenberg H. 1979. *Zeigerwerte der Gefäßpflanzen Mitteleuropas: indicator values of vascular plants in Central Europe.* Goettingen, Germany: Goltze.

Fischer C, Tischer J, Roscher C, Eisenhauer N, Ravenek J, Gleixner G, Attinger S, Jensen B, de Kroon H, Mommer L *et al.* 2015. Plant species diversity affects infiltration capacity in an experimental grassland through changes in soil properties. *Plant and Soil* 397: 1–16.

Fischer FM, Wright AJ, Eisenhauer N, Ebeling A, Roscher C, Wagg C, Weigelt A, Weisser WW, Pillar VD. 2016. Plant species richness and functional traits affect community stability after a flood event. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* 371: 20150276–20150278.

Fornara DA, Tilman D. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *Journal of Ecology* 96: 314–322.

Fraaije RGA, ter Braak CJF, Verduyn B, Breeman LBS, Verhoeven JTA, Soons MB. 2015. Early plant recruitment stages set the template for the development of vegetation patterns along a hydrological gradient (A Brody, Ed.). *Functional Ecology* 29: 971–980.

Garssen AG, Baattrup-Pedersen A, Voesenek LACJ, Verhoeven JTA, Soons MB. 2015. Riparian plant community responses to increased flooding: a meta-analysis. *Global Change Biology* 21: 2881–2890.

Gotelli NJ, Ellison AM. 2012. *A primer of ecological statistics.* Sunderland, MA, USA: Sinauer Associates.

Gubsch M, Buchmann N, Schmid B, Schulze ED. 2011. Differential effects of plant diversity on functional trait variation of grass species. *Annals of Botany* 107: 157–169.

Hayot Carbonero C, Mueller-Harvey I, Brown TA, Smith L. 2011. Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. *Plant Genetic Resources* 9: 70–85.

Heinrichs DH. 1970. Flooding tolerance of legumes. *Canadian Journal of Plant Science* 50: 435–438.

Hirabayashi Y, Mahendran R, Koirala S, Konoshima L, Yamazaki D, Watanabe S, Kim H, Kanae S. 2013. Global flood risk under climate change. *Nature Climate Change* 3: 816–821.

Huber H, Jacobs E, Visser EJW. 2008. Variation in flooding-induced morphological traits in natural populations of white clover (*Trifolium repens*) and their effects on plant performance during soil flooding. *Annals of Botany* 103: 377–386.

Isbell F, Craven D, Connolly J, Loreau M, Schmid B, Beierkuhnlein C, Bezemer TM, Bonin C, Bruelheide H, De Luca E *et al.* 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature Communications* 526: 574–577.

James EK, Crawford R. 1998. Effect of oxygen availability on nitrogen fixation by two *Lotus* species under flooded conditions. *Journal of Experimental Botany* 49: 599–609.

Jongman B, Hochrainer-Stigler S, Feyen L, Aerts JCJH, Mechler R, Botzen WJW, Bouwer LM, Pflug G, Rojas R, Ward PJ. 2014. Increasing stress on disaster-risk finance due to large floods. *Nature Climate Change* 4: 264–268.

Kay BD. 1996. Soil structure and organic carbon: a review. In: Lal R, Kimble JM, Follett RF, Stewart BA, eds. *Soil processes and the carbon cycle.* Boca Raton, FL, USA: CRC Press, 169–198.

Lange M, Eisenhauer N, Sierra CA, Bessler H. 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* 6: 6707.

Mielke MS, de Almeida A-AF, Gomes FP, Aguilar MAG, Mangabeira PAO. 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. *Environmental and Experimental Botany* 50: 221–231.

Mommer L, de Kroon H, Pierik R, Bogemann GM, Visser EJW. 2005. A functional comparison of acclimation to shade and submergence in two terrestrial plant species. *New Phytologist* 167: 197–206.

Mommer L, Lenssen JPM, Huber H, Visser EJW, De Kroon H. 2006. Ecophysiological determinants of plant performance under flooding: a comparative study of seven plant families. *Journal of Ecology* 94: 1117–1129.

Mommer L, Visser E. 2005. Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. *Annals of Botany* 96: 581–589.

Morris J, Brewin P. 2014. The impact of seasonal flooding on agriculture: the spring 2012 floods in Somerset, England. *Journal of Flood Risk Management* 7: 128–140.

Mueller KE, Tilman D, Fornara DA, Hobbie SE. 2013. Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. *Ecology* 94: 787–793.

Mulder C, Jumpponen A, Hogberg P, Huss-Danell K. 2002. How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia* 133: 412–421.

Mylona P, Pawlowski K, Bisseling T. 1995. Symbiotic nitrogen fixation. *Plant Cell* 7: 869–885.

Odum EP, Finn JT, Franz EH. 1979. Perturbation theory and the subsidy-stress gradient. *BioScience* 29: 349–352.

Pérès G, Cluzeau D, Menasseri S, Soussana J-F, Bessler H, Engels C, Habekost M, Gleixner G, Weigelt A, Weisser WW *et al.* 2013. Mechanisms linking plant community properties to soil aggregate stability in an experimental grassland plant diversity gradient. *Plant and Soil* 373: 285–299.

Ravenek JM, Bessler H, Engels C, Scherer-Lorenzen M, Gessler A, Gockele A, De Luca E, Temperton VM, Ebeling A, Roscher C *et al.* 2014. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. *Oikos* 123: 1528–1536.

Reich P, Knops J, Tilman D, Craine J, Ellsworth D, Tjoelker M, Lee T, Wedin D, Naeem S, Bahauddin D. 2001. Plant diversity enhances ecosystem responses to elevated $CO_2$ and nitrogen deposition. *Nature Communications* 410: 809–810.

Reich PB, Tilman D, Isbell F, Mueller K, Hobbie SE, Flynn DFB, Eisenhauer N. 2012. Impacts of biodiversity loss escalate through time as redundancy fades. *Science* 336: 589–592.

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze ED. 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* 5: 107–121.

Sasidharan R, Voesenek LACJ. 2015. Ethylene-mediated acclimations to flooding stress. *Plant Physiology* 169: 3–12.

Silvertown J, Dodd ME, Gowing D, Mountford JO. 1999. Hydrologically defined niches reveal a basis for species richness in plant communities. *Nature Communications* 400: 61–63.

Sněhota M, Císlerová M, Amin MHG, Hall LD. 2010. Tracing the entrapped air in heterogeneous soil by means of magnetic resonance imaging. *Vadose Zone Journal* 9: 373–384.

Stocker TF, Qin D, Plattner GK, Alexander LV, Allen SK, Bindoff NL, Breon FM, Church JA, Cubasch U, Emori S *et al.* 2013. In: Stocker RF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, eds. Technical summary. In: *Climate change 2013: the physical science basis. Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change.* Cambridge, UK & New York, NY, USA: Cambridge University Press.

Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E. 1997. The influence of functional diversity and composition on ecosystem processes. *Science* 277: 1300.

Toogood SE, Joyce CB, Waite S. 2007. Response of floodplain grassland plant communities to altered water regimes. *Plant Ecology* 197: 285–298.

Van Eck W, Van de Steeg HM, Blom C, De Kroon H. 2004. Is tolerance to summer flooding correlated with distribution patterns in river floodplains? A comparative study of 20 terrestrial grassland species. *Oikos* 107: 393–405.

Vashisht D, Hesselink A, Pierik R, Ammerlaan JMH, Bailey-Serres J, Visser EJW, Pedersen O, van Zanten M, Vreugdenhil D, Jamar DCL *et al.* 2010. Natural variation of submergence tolerance among *Arabidopsis thaliana* accessions. *New Phytologist* 190: 299–310.

Vervuren P, Blom C, De Kroon H. 2003. Extreme flooding events on the Rhine and the survival and distribution of riparian plant species. *Journal of Ecology* 91: 135–146.

Violle C, Bonis A, Plantegenest M, Cudennec C, Damgaard C, Marion B, Le Cœur D, Bouzillé J-B. 2010. Plant functional traits capture species richness variations along a flooding gradient. *Oikos* 120: 389–398.

Visser EJW, Bogemann GM. 2003. Measurement of porosity in very small samples of plant tissue. *Plant and Soil* 253: 81–90.

Visser E, Bögemann GM, Van de Steeg HM. 2000. Flooding tolerance of *Carex* species in relation to field distribution and aerenchyma formation. *New Phytologist* 148: 93–103.

Voesenek LACJ, Bailey-Serres J. 2015. Flood adaptive traits and processes: an overview. *New Phytologist* 206: 57–73.

Voesenek LACJ, Rijnders JHGM, Peeters AJM, Van de Steeg HM, De Kroon H. 2004. Plant hormones regulate fast shoot elongation under water: from genes to communities. *Ecology* 85: 16–27.

Votrubová J, Císlerová M, Gao Amin MH, Hall LD. 2003. Recurrent ponded infiltration into structured soil: a magnetic resonance imaging study. *Water Resources Research* 39: 1–12.

Winkel A, Visser EJW, Colmer TD, Brodersen KP, Voesenek LACJ, Sand-Jensen K, Pedersen O. 2016. Leaf gas films, underwater photosynthesis and plant species distributions in a flood gradient. *Plant Cell and Environment* 39: 1537–1548.

Wright AJ, Ebeling A, De Kroon H, Roscher C, Weigelt A, Buchmann N, Buchmann T, Fischer C, Hacker N, Hildebrandt A *et al.* 2015b. Flooding disturbances increase resource availability and productivity but reduce stability in diverse plant communities. *Nature Communications* 6: 1–6.

Wright A, Schnitzer SA, Reich PB. 2015a. Daily environmental conditions determine the competition–facilitation balance for plant water status. *Journal of Ecology* 103: 648–656.

## Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Flooding index at the site compared with maximum water depth.

**Fig. S2** Biomass sampling, mowing, and growth indices over time.

**Fig. S3** Species-specific harvest data from every harvest between May 2010 and September 2013.

**Fig. S4** Species-specific log response ratios in monoculture and mixture compared with longer-term average (2010–2012).

**Fig. S5** Aerenchyma ranking classification.

**Fig. S6** Multivariate PCA analysis including all of the physiological traits and performance in July 2013 and September 2013.

**Fig. S7** Average height of plants within each of the four functional groups (grasses, legumes, small herbs, and tall herbs).

**Fig. S8** Species-specific height and emergence above the flood waters in each of the plots in the main experiment on June 7.

**Fig. S9** Starch content of all plants in all functional groups growing in monocultures.

**Fig. S10** The effect of flooding index on soil air porosity (24 June 2013).

**Table S1** Temperature and precipitation data during the year of the flood and compared with the 3 yr average and the 10 yr average

**Table S2** All traits, biomass, and log response ratios for all species assessed in the current analysis

**Table S3** Stepwise regression with forward selection criteria to determine the mechanisms behind monoculture vs mixture effects in July 2013

**Table S4** Full mixed-effects models using factors determined with stepwise regression for each time period, including plot diversity and all selected variables in the model

**Table S5** Stepwise regression with forward selection criteria to determine the mechanisms behind monoculture vs mixture effects in September 2013

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.