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Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought

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Summary

1. Due to climate change, an increasing frequency and severity of drought events are expected to impair grassland productivity, particularly of intensively managed temperate grasslands.

2. To assess drought impacts, a common field experiment to manipulate precipitation was set up at three sites (two Swiss and one Irish) using monocultures and mixtures with two and four key forage species. Species differed in their functional traits: a shallow-rooted non-legume (*Lolium perenne* L.), a deep-rooted non-legume (*Cichorium intybus* L.), a shallow-rooted legume (*Trifolium repens* L.) and a deep-rooted legume (*Trifolium pratense* L.). A 9-week summer drought was simulated, and soil water status, above-ground biomass yield and plant nitrogen (N) limitation were compared to a rainfed control.

3. Based on soil water measurements, the drought induced severe stress at both Swiss sites and extreme stress at the Irish site. Under severe stress, the legumes were more drought resistant and showed an average change in above-ground biomass (CAB, compared to rainfed control) of only −8% and −24% (for the two Swiss sites), while the non-legumes had an average CAB of −51% and −68%. The lower resistance of non-legumes coincided with an apparent limitation of plant N, which further increased under drought. Under extreme drought (Irish site), growth nearly ceased with an average CAB of −85%.

4. During a 6-week post-drought period with adequate water supply (Swiss sites), formerly drought-stressed species were highly resilient and either attained (legumes) or clearly outperformed (non-legumes) the yield level of the rainfed controls. This outperformance coincided with post-drought reductions in N limitation in formerly drought-stressed species. As a result, aggregated over the drought and the post-drought periods, a negative drought impact was found only for the shallow-rooted *L. perenne* at one of the severely stressed sites.

5. Significant overyielding by multispecies mixtures was evident under rainfed control conditions (+38% across all three sites, $P < 0{\cdot}05$) and was equally apparent under severe drought (+50%, $P < 0{\cdot}05$). This overyielding was greatest in mixtures with approximately equal species proportions and was sufficiently large that drought-stressed mixtures at least attained the same yield as the average of the rainfed monocultures. Under extreme drought, growth almost ceased in monocultures and mixtures.

6. *Synthesis and applications*. Yields of selected species of intensively managed temperate grasslands are either resistant to a single severe drought or are highly resilient as soon as soil moisture levels recover after the drought event. However, these forage species seem unable to

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cope with an extreme drought event. Combining species in mixtures can compensate for yield reductions caused by severe drought and it offers a practical management tool to adapt forage production to climate change.

Key-words: *Cichorium intybus*, climate change, drought adaptation, forage mixtures, *Lolium perenne*, nitrogen limitation, post-drought recovery, precipitation manipulation experiment, *Trifolium pratense*, *Trifolium repens*

Introduction

Extreme weather events are predicted to occur more frequently due to climate change (Orlowsky & Seneviratne 2012). In temperate regions, drought events are expected to increase in frequency and severity (Seneviratne *et al.* 2012). Whereas plants can withstand moderate changes in total annual precipitation, increased variability in precipitation and the amount of precipitation per event, for example prolonged periods of drought or waterlogging, can substantially impair above-ground biomass production (Swemmer, Knapp & Snyman 2007).

Drought events can affect above-ground biomass yield of grasslands very differently depending on site conditions such as soil type, pre-drought climatic conditions and the intensity of management (Gilgen & Buchmann 2009; Vicca *et al.* 2012). Whereas grassland of low productivity can be quite resistant to drought events (Jentsch *et al.* 2011; Hoeppner & Dukes 2012), intensively managed, highly productive grassland may be more susceptible with its specialized and high yielding forage species. A possible reason for increased drought susceptibility may be found in the high cutting frequency in intensive grassland systems (up to six harvests per year). Indeed, a substantial impairment of biomass yield under drought with increasing productivity and cutting frequency was found in a study that evaluated intensive grassland (Vogel, Scherer-Lorenzen & Weigelt 2012). On the economic side, in Switzerland, for example, the financial loss due to drought events is distinctively higher for intensively managed compared to less intensively or extensively managed grassland (Finger *et al.* 2013). Nevertheless, recent experiments investigating drought effects have been performed mainly on less productive grassland (e.g. Jentsch *et al.* 2011; Hoeppner & Dukes 2012) or on grassland dominated by shallow-rooted grasses (Gilgen & Buchmann 2009).

Resistance of a managed grassland to drought could be enhanced by using a targeted selection of species with functional traits that allow them to cope with drought stress. In this context, resistance is defined as the degree of impairment during a drought event (Pimm 1984). Deep-rooted grassland species showed an increased drought resistance (Skinner, Gustine & Sanderson 2004), probably due to their ability to acquire water from deeper soil layers and are therefore often discussed as an adaption option to drought. Legumes might resist drought events because they are able to utilize atmospheric dinitrogen (N_2) for their N requirements. This could be a valuable trait where plant-available soil N is increasingly constrained under drought (Durand, Gonzalez-Dugo & Gastal 2010). However, concluding from experiments in growth chambers, symbiotic N_2 fixation is restricted under water limitation (e.g. Serraj, Sinclair & Purcell 1999), and there is a need to investigate the ability of forage legumes to resist severe droughts under field conditions.

High resilience of managed grassland [i.e. fast and complete recovery during a period of adequate water supply following a drought event (*sensu* Pimm 1984)], is crucial to minimize drought-induced losses and to ensure long-term yield stability (Zwicke *et al.* 2013). Grasslands of low-to-medium management intensity have shown resilience to drought (Vogel, Scherer-Lorenzen & Weigelt 2012; Hoover, Knapp & Smith 2014). Under intensive grassland management, high resilience is especially important as high annual yields are expected with several harvests per year, potentially including one or more harvests following the drought event within the same year. The extent of loss of production and income for farmers caused by a drought event can therefore reasonably be defined as the total forage loss starting with the drought event and ending with the growing season (or as long as drought effects persist), thus including losses due to a lack of resilience after cessation of drought. Consequently, resilience to drought should receive the same attention as resistance to drought itself.

Combining selected species in grassland mixtures under ambient climatic conditions can result in overyielding of above-ground biomass production (mixture performance greater than the weighted average of the respective monocultures) due to niche complementarity and positive interspecific interactions (Kirwan *et al.* 2007). High overyielding has recently been demonstrated in intensively managed grass–legume mixtures over largely differing climatic zones from Mediterranean to Nordic regions (Finn *et al.* 2013). However, it remains to be tested whether such mixture advantage is still evident under environmental stress, for example drought conditions. If so, the yield gain due to overyielding of mixtures affected by drought could compensate for the yield loss of monocultures due to drought.

Here, we investigate the drought resistance and post-drought resilience of four key species from intensively managed grassland, selected for their distinct functional traits. Rainout shelters were installed for 9 weeks over monocultures and mixtures with two and four species, and a common design was implemented at two Swiss and one Irish site so as to achieve high experimental similarity across sites. The common methods are important because there is a lack of adequate comparisons of precipitation manipulation experiments in a multi-site framework (Vicca *et al.* 2012). Special attention was paid to quantify drought severities of the sites by evaluating soil water status. The following specific hypotheses were addressed:

1. Deep-rooted species are more resistant to drought than shallow-rooted species, legumes are more resistant to drought than non-legumes, and such resistance is negatively correlated with (a) drought severity and (b) plant N limitation.

2. During the post-drought period, non-resistant species are resilient, and their yields attain the levels in non-drought conditions.

3. Mixtures perform better than the average of monocultures (overyielding) under rainfed control as well as under drought conditions and therefore have the potential to compensate for the drought impact on species grown in monocultures.

Materials and methods

EXPERIMENTAL SITES

A common field experiment to manipulate precipitation was established at three sites using a similar design to achieve as high experimental equivalence across sites as possible. Namely, we used at all sites the simplex design (Cornell 2002) and the same plant species, applied the same length of drought stress during the summer months (July and August) and investigated two regrowths under drought. There were some technical differences across sites, for example regarding total amount of N fertilizer applied and number of cuts, partly owing to specific local conditions. Sites were in Switzerland at Tänikon (47°28′41″N, 8°54′25″E, 538 m a.s.l.) and at Reckenholz (47°26′12″N, 8°31′51″E, 479 m a.s.l.) and in Ireland at Wexford (52°17′35″N, 6°30′8″E, 58 m a.s.l.). The experiment was located on a soil classified as brown earth with no influence of the ground water-table at all three sites. Soil texture and pH of the top soil varied among the Irish and Swiss sites (Tänikon-CH: 26% sand, 45% silt, 29% clay, pH = 7·0; Reckenholz-CH: 32% sand, 42% silt, 26% clay, pH = 7·1; Wexford-IE: 56% sand, 32% silt, 12% clay, pH = 4·9) allowing us to relate the effects of soil texture to drought response. To enable the evaluation of a fully established system, plots were established in the year prior to investigation and measurements were taken in 2011 at Tänikon-CH (mean annual temperature: 9·7 °C, annual precipitation: 970 mm), in 2012 at Reckenholz-CH (9·8 °C, 1165 mm) and in 2013 at Wexford-IE (10·0 °C, 888 mm).

EXPERIMENTAL DESIGN

Four perennial species, representative of intensively managed temperate grassland systems and used as key forage species in ruminant production, were selected for experimentation based on the factorial combination of their specific functional traits related to rooting depth and N acquisition (non-fixing for non-legumes, N_2 fixing for legumes): a shallow-rooted non-legume (the grass *Lolium perenne* L., cultivar (cv.) Alligator), a deep-rooted non-legume (the forb *Cichorium intybus* L., cv. Puna II), a shallow-rooted legume (*Trifolium repens* L., cv. Hebe) and a deep-rooted legume (*Trifolium pratense* L., cv. Pastor at Tänikon-CH and cv. Dafila at the two other sites). Using these four species, plots of 5 × 3 m were established in monocultures and mixtures of varying species richness and sowing proportions following a simplex design (Cornell 2002; Kirwan *et al.* 2009): monocultures (100% of one species), binary mixtures (50% of each of the two species), an equi-proportional mixture (25% of each of the four species) and dominant mixtures (79% of the dominant species, 7% of each of the other three species; see Table S1 in Supporting Information). Monocultures and mixtures were established as control treatment under ambient rainfed conditions and as drought treatment where a summer drought event was simulated (see below). Full details to the design are given in Appendix S1.

A summer drought event of 9 weeks was simulated at each site with precipitation being excluded completely from the drought treatment using rainout shelters. The tunnel-shaped shelters consisted of steel frames (3 × 5·5 m and a height of 140 cm) covered with a transparent and ultraviolet light transmissible plastic foil (Gewächshausfolie UV5, 200 µm, Folitec Agrarfolien-Vertrieb, Germany, at Tänikon-CH and Reckenholz-CH; SunMaster SuperThermic, 150 µm, XL Horticulture, UK, at Wexford-IE). Shelters were open at both opposing ends and had a ventilation opening of 35 cm over the entire length at the top and at both sides at the bottom to stimulate air circulation (Fig. S1), and thus, temperature increase underneath the shelters was minor (0·5–0·9 °C; Table S2, Fig. S2). At Reckenholz-CH, we had to restart the drought period after 5 weeks due to a heavy thunderstorm. The 9-week length of the drought period was chosen to simulate a strong drought event spanning two entire regrowth periods. Our drought treatment reduced summer precipitation (June, July and August) by 66% (average across sites, Table S2; see Appendix S1 for an assessment of drought severity). The 6-week regrowth period (7 weeks at Wexford-IE) following the drought was defined as the post-drought period during which the resilience of species was examined.

All plots of a site received the same amount of mineral N fertilizer: 145 kg N ha^{-1} $year^{-1}$ at Tänikon-CH (divided into four applications), 200 kg N ha^{-1} $year^{-1}$ at Reckenholz-CH (six applications) and 130 kg N ha^{-1} $year^{-1}$ at Wexford-IE (four applications). Phosphorus and potassium were applied following local fertilization recommendations for intensively managed grassland. Details to sward establishment and total annual biomass yields are provided in Appendix S1.

MEASUREMENTS

Information on precipitation was provided by national meteorological services at a maximum distance of 1400 m from each site. Soil moisture content was measured hourly in equi-proportional mixtures at 5 cm and 40 cm soil depth under drought and control (at Tänikon-CH: $n = 2$ per depth, EC-5 sensor, Decagon, USA; at Reckenholz-CH: $n = 3$ per depth, 5TM sensor, Decagon, USA) and was measured weekly at 10 cm and 40 cm at Wexford-IE ($n = 3$ per depth, PR2 Probe, Delta-T Devices, Cambridge, UK). Soil desorption curves (the relationship between soil water content and soil matric potential) were determined for each site ($n = 6$ per soil depth) using a standardized pressure plate method (Agroscope Reckenholz-Tänikon ART 2012). This provided a common metric for the physical soil environment to quantify water stress. We refer to a soil matric potential of −1·5 MPa because this is the approximate threshold of plant accessible soil water.

Plots were cut five times per year (plus two clearing cuts) at Tänikon-CH, six times at Reckenholz-CH and five times at Wexford-IE (see Table S3 for harvest dates). Biomass yield was measured from a central strip of 5 × 1·5 m using an experimental plot harvester (CH: Hege 212, Wintersteiger, Ried im Innkreis, Austria; IE: Haldrup plot combine, HALDRUP, Ilshofen, Germany). Dry matter content of each plot yield was determined by drying a subsample of bulk mass at 100 °C for 24 h. Plant N concentration of above-ground biomass of monocultures was measured at the end of the drought and the end of the post-drought period (see Appendix S1 for details on the analysis of plant N concentration).

DATA ANALYSIS

We refer to resistance as the species' biomass response under drought compared to rainfed control conditions during the drought period and to resilience as the biomass response of formerly drought-stressed stands compared to former non-stressed control stands during the post-drought period.

To compare above-ground dry matter yield (DMY) between the rainfed control (Ctr) and drought (Drt) treatment at individual harvests, we first calculated the response ratio (R) for each monoculture and harvest as:

$$R = \text{DMY Drt}/\text{DMY Ctr} \qquad \text{eqn 1}$$

Because it is desirable to draw statistical inference on the natural logarithm of a response ratio, the log response ratio L (= ln(R)) was used for statistical analyses with standard deviation (SD) following Hedges, Gurevitch & Curtis (1999):

$$\text{SD} = \sqrt{\frac{\text{SD}^2_{\text{DMY Ctr}}}{n * \text{Mean}^2_{\text{DMY Ctr}}} + \frac{\text{SD}^2_{\text{DMY Drt}}}{n * \text{Mean}^2_{\text{DMY Drt}}}} \qquad \text{eqn 2}$$

Mean_{DMY} is the average across the number of sample replicates (n) per treatment. For all monocultures, L was analysed by analysis of variance with species (factor of four levels) as main effect, and inference on differences among factor levels was derived from the models' contrasts. For clarity, percentage of change in above-ground biomass (% CAB) is presented in figures and text:

$$\text{CAB}\ (\%) = 100 * (R - 1) \qquad \text{eqn 3}$$

Overyielding of mixtures under rainfed control and drought conditions was analysed using a regression-based approach following Kirwan *et al.* (2009) (see Appendix S1). Total stand DMY was regressed on the sown proportional contributions of the four species and the drought treatment, as follows:

$$\begin{aligned}\text{DMY} = {} & \beta_1 P_{\text{Lp}} + \beta_2 P_{\text{Ci}} + \beta_3 P_{\text{Tr}} + \beta_4 P_{\text{Tp}} + \delta_1 D_{\text{SR*DR}} + \delta_2 D_{\text{NL*LE}} \\ & + \delta_3 D_{\text{CROSS}} + \gamma_1 P_{\text{Lp}}\text{Drt\_Treat} + \gamma_2 P_{\text{Ci}}\text{Drt\_Treat} \\ & + \gamma_3 P_{\text{Tr}}\text{Drt\_Treat} + \gamma_4 P_{\text{Tp}}\text{Drt\_Treat} + \varepsilon\end{aligned} \qquad \text{eqn 4}$$

where P represents the sown species proportions (Lp = *L. perenne*, Ci = *C. intybus*, Tr = *T. repens*, Tp = *T. pratense*) in a stand. The identity effect of each species is thus estimated by β_1–β_4, and, if $P = 1$, β coefficients estimate DMY of a species grown in monoculture. Pairwise interactions between species were grouped together to specifically reveal diversity effects (D) between shallow- and deep-rooted species (SR*DR), between non-legumes and legumes (NL*LE) and effects due to mixing the shallow-rooted non-legume with the deep-rooted legume and *vice versa* (CROSS) (detailed in Appendix S1). The coefficients δ_1 to δ_3 therefore estimate the diversity effect (i.e. net interactions) between species groups specified by functional traits. The effect of the drought treatment (Drt_Treat: factor with two levels: 0 for control, 1 for drought) is estimated by coefficients γ_1–γ_4. To account for the split-plot structure of the design at Reckenholz-CH and Wexford-IE, eqn 4 was extended to a linear mixed model (Pinheiro & Bates 2009) by specifying each pair of rainfed control and drought plots as a random unit (modelled as random intercept).

Plant N limitation was determined as the difference between the measured plant N concentration and the critical N concentration (CNC) that C_3 species need for N-unlimited growth. The CNC has been validated for a wide range of forage grass and non-grass species including legumes and has been defined by Lemaire & Gastal (1997) as:

$$\text{CNC} = 4{\cdot}8\,\text{DMY}^{-0{\cdot}32} \qquad \text{eqn 5}$$

CNC was kept constant at 4·8% for DMY < 1 t ha^{-1} to consider the absence of competition between individual plants under low biomass yield (Lemaire & Gastal 1997). Differences in plant N limitation between the rainfed control and drought treatment in monocultures were analysed using a linear mixed model with species and drought treatment as main effects, including their interaction, and a random term as described for eqn 4.

We defined the cumulative drought effect as the difference in above-ground biomass yield between ambient and drought conditions aggregated over the two regrowths during drought and the regrowth during the post-drought period. Differences in these cumulative yields between the rainfed control and drought treatment were analysed in the same way as described for plant N limitation. All analyses were performed with the statistics software R (R Core Team 2016).

Results

DROUGHT SEVERITY DIFFERED ACROSS SITES

The simulated 9-week summer drought induced different changes in the soil water status depending on site and soil depth (Fig. 1). Under drought conditions, the critical soil matric potential of −1·5 MPa (representing the approximate threshold of plant accessible soil water) was reached persistently at both depths and all sites except at Tänikon-CH at 40 cm depth (Fig. 1d). Due to an extraordinarily dry summer at Wexford-IE (only 63% of precipitation compared to the 30 year average, Table S2), the threshold of −1·5 MPa was reached at 10 cm depth under both drought and rainfed control conditions even prior to

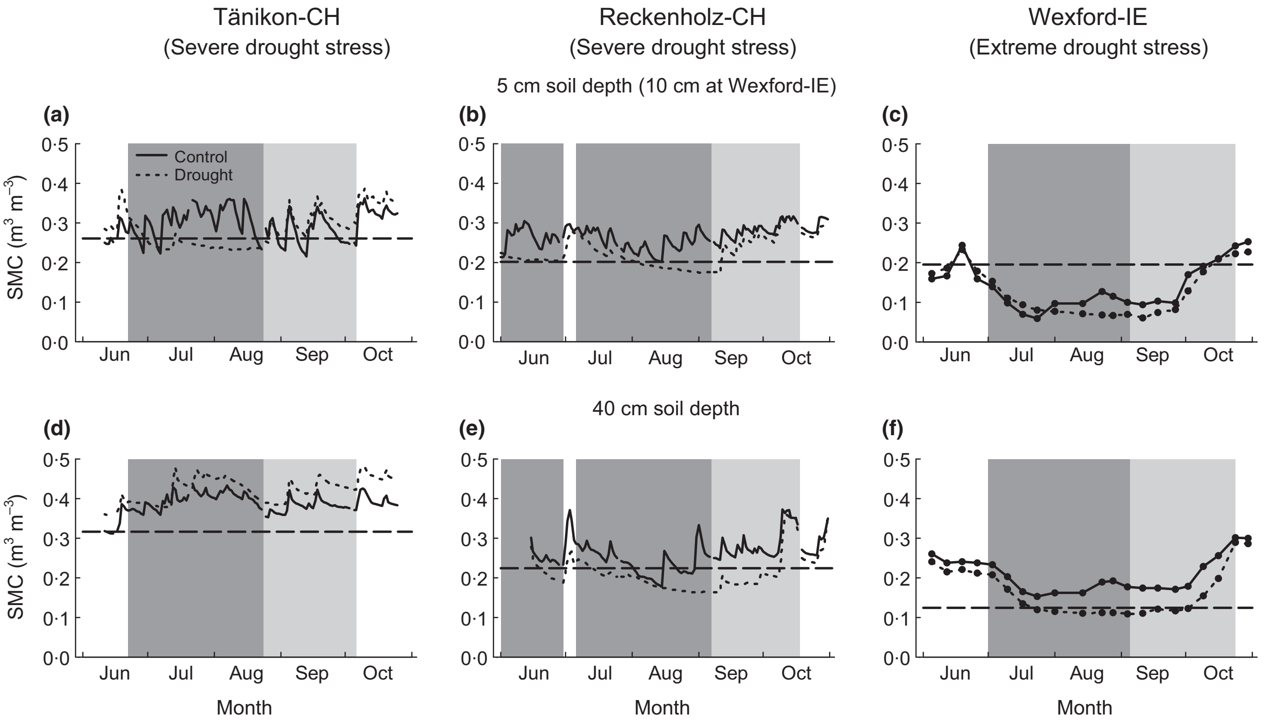

Fig. 1. Soil moisture content (SMC) at 5 cm (10 cm at Wexford-IE) (a–c) and at 40 cm soil depth (d–f) of rainfed control and drought plots of the equi-proportional mixture during the drought period (dark grey shaded) and the subsequent post-drought period (light grey shaded) at the two severely stressed sites Tänikon-CH (a and d) and Reckenholz-CH (b and e) and the extremely stressed site Wexford-IE (c and f). Displayed are means of SMC per treatment and site ($n = 2$ at Tänikon-CH, $n = 3$ at Reckenholz-CH and Wexford-IE). The dashed horizontal line (– – – –) is the SMC corresponding to a soil matric potential of −1·5 MPa, which is the approximate threshold of plant accessible soil water.

the experimental drought and dropped far below this threshold during most of the drought period (Fig. 1c), in contrast to the Swiss sites. Thus, the induced drought stress can be considered to be severe at both Swiss sites, but extreme at the Irish site.

After the drought period, soil moisture content of the formerly sheltered plots generally reached the level of the rainfed control treatment at both Swiss sites and was in the range of adequate water availability at least at 5 cm soil depth (Fig. 1). In contrast, at Wexford-IE, soil moisture content remained below the matric potential of −1·5 MPa in formerly sheltered plots until close to the end of the post-drought period at both soil depths (Fig. 1c,f), due to a lack of precipitation (Fig. S2; there was no artificial irrigation).

DROUGHT RESISTANCE DEPENDED ON FORAGE SPECIES AND DROUGHT SEVERITY

At the mid-drought harvest (first regrowth under drought), there was generally no significant change in above-ground biomass (CAB) due to drought (Fig. 2), except for the two non-legumes at Tänikon-CH (*L. perenne* −46% CAB, $P = 0{\cdot}002$; *C. intybus*: −27% CAB, $P = 0{\cdot}072$). In the harvest at the end of the drought period, above-ground biomass yield was significantly reduced under drought for the two non-legumes at both severely stressed sites Tänikon-CH (−51% on average, $P \leq 0{\cdot}046$) and Reckenholz-CH (−68%, $P < 0{\cdot}001$), while the two legumes *T. repens* and *T. pratense* largely resisted the drought stress (−8% CAB, $P > 0{\cdot}485$, Tänikon-CH; −24% CAB, $P > 0{\cdot}124$, Reckenholz-CH, Fig. 2a,b). Consequently, CAB of legumes was significantly smaller than that of non-legumes ($P \leq 0{\cdot}003$). In contrast, under extreme drought stress at Wexford-IE, the deep-rooted non-legume *C. intybus* was least impaired (−57% CAB, $P = 0{\cdot}021$), whereas the yield of both legumes completely collapsed (−98% CAB, $P < 0{\cdot}001$, Fig. 2c).

RESILIENCE DEPENDED ON FORAGE SPECIES AND POST-DROUGHT WATER SUPPLY

After a post-drought period of 6 weeks with adequate water supply (Swiss sites), the biomass yields of both formerly drought-stressed legumes reached levels of their rainfed controls (+18% CAB, Tänikon-CH; −2% CAB, Reckenholz-CH; Fig. 2). Remarkably, both formerly drought-stressed non-legumes revealed a substantial and positive CAB during the post-drought period at Tänikon-CH (+87%, $P \leq 0{\cdot}025$) and Reckenholz-CH (+62%, $P \leq 0{\cdot}030$) and thus clearly outperformed the controls. Consequently, CAB of non-legumes was significantly greater than that of legumes ($P \leq 0{\cdot}015$ for both Swiss sites). Under the specific conditions of Wexford-IE,

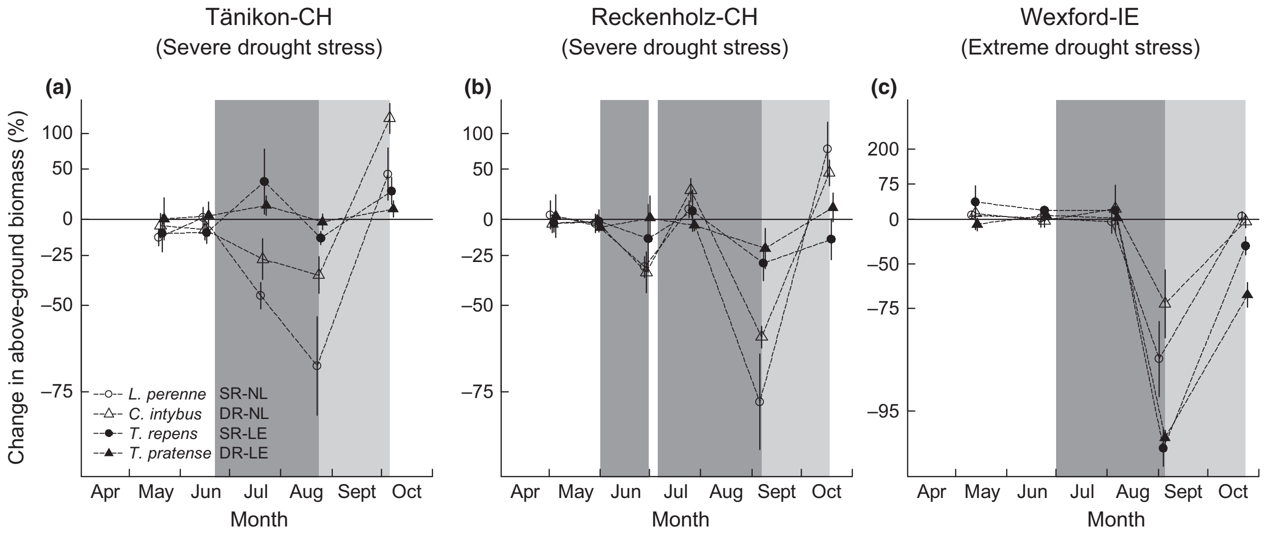

Fig. 2. Seasonal trajectory of change in above-ground biomass (CAB, displayed on log-scale) during a simulated summer drought event (dark grey shaded) and the subsequent post-drought period (light grey shaded) of monocultures of four species over one growing season from April to October for five harvests at Tänikon-CH (a), for six harvests at Reckenholz-CH (b) and for five harvests at Wexford-IE (c) (see Table S3 for harvest dates). CAB (%) = 100 * $\left(\frac{\text{DMY Drought}}{\text{DMY Control}} - 1\right)$; displayed is the mean CAB ± 1 SD ($n = 3$). Note the different y-axes. At Reckenholz-CH, the drought period had to be restarted after a heavy thunderstorm at the end of June. SR: shallow-rooted, DR: deep-rooted, NL: non-legume, LE: legume.

T. repens and *T. pratense* were the only two species that did not recover completely (−51% CAB on average, $P \leq 0{\cdot}004$, Fig. 2c). This was most probably because these species were heavily impaired by the extreme drought, which continued during most of the post-drought period due to the lack of precipitation (see Fig. 1).

DROUGHT SEVERITY DETERMINED THE CUMULATIVE DROUGHT EFFECT

Due to the high resistance of legumes and the high resilience of non-legumes (Fig. 2), the aggregated above-ground biomass from the start of the drought until the end of the post-drought period was in general little impaired by the drought event, and there was no clear pattern regarding rooting depth and N acquisition (Fig. 3). At both severely stressed sites, a negative cumulative drought effect was only apparent for the shallow-rooted non-legume *L. perenne* at Tänikon-CH (−40% CAB, Fig. 3a). Under the extremely stressed conditions at Wexford-IE, where the drought stress persisted throughout most of the post-drought period, the two legumes showed a negative cumulative CAB of −28% and −27%, respectively (Fig. 3c). Even under these extreme conditions, *L. perenne* and *C. intybus* revealed no negative cumulative drought effect.

SIGNIFICANT OVERYIELDING ALSO OCCURRED UNDER SEVERE DROUGHT

Growing all four species in mixture under rainfed control conditions resulted in substantial overyielding of +38% (averaged over all sites), and this overyielding was equally apparent under severe drought at the Swiss sites (+50%) (Table 1, Fig. 4, Table S4). Under extreme drought at Wexford-IE, growth almost ceased in all stands (Table 1, Fig. 4c). Overyielding was largely driven by mixing legumes and non-legumes, and by the crossed trait combination where the shallow-rooted non-legume was combined with the deep-rooted legume and *vice versa* (Table 1, diversity effects). Most remarkably, overyielding of severely drought-stressed mixtures (Swiss sites) was so strong that stands close to the equi-proportional mixture reached the same yield as the average of monocultures under rainfed control conditions (Fig. 4a,b, around 50% legume proportion). Thus, despite mixtures being affected by drought, the yield advantage from mixing species could compensate for the drought impairment and attain the average yield of the rainfed monocultures.

PLANT NITROGEN LIMITATION IN NON-LEGUMES

Both legumes were not or only little N limited, and their N status did not generally respond to drought (Fig. 5). In contrast, both non-legumes were clearly N limited under rainfed control and drought conditions. Importantly, the drought effect resulted in a substantially increased N limitation in the deep-rooted non-legume *C. intybus* at both Swiss sites (Fig. 5a,b); however, during the post-drought period, the drought effect was inversed and resulted in a decrease in N limitation in non-legume species (Fig. 5d,e).

Discussion

We investigated the resistance and resilience to drought of agronomically important species of productive temperate

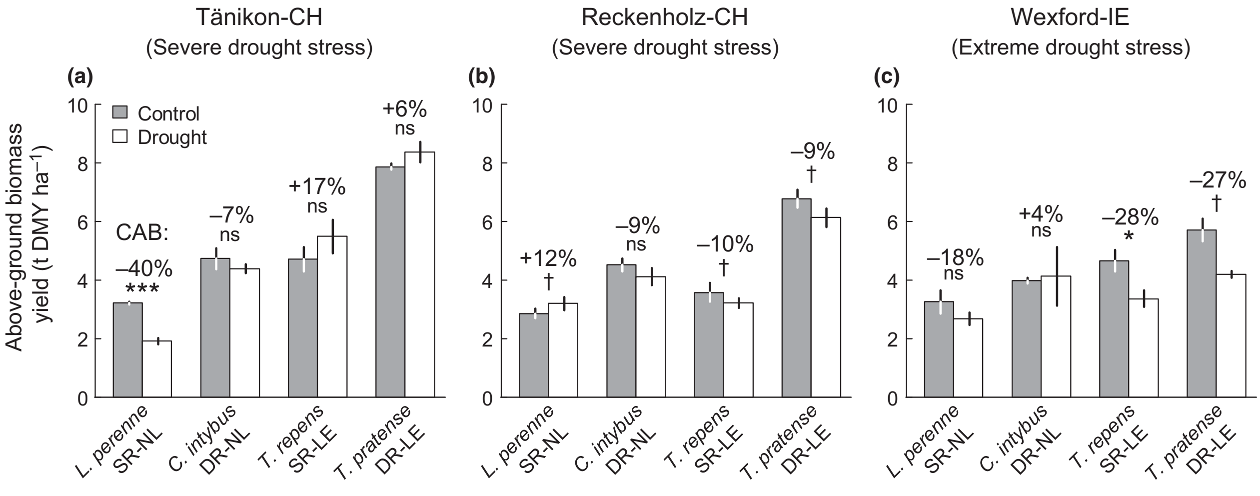

Fig. 3. Cumulative drought effect on above-ground biomass yield of the four species in monocultures under rainfed control and drought conditions at Tänikon-CH (a), Reckenholz-CH (b) and Wexford-IE (c). Yields were aggregated over both harvests during the drought period and the harvest at the end of the post-drought period. Displayed are means ± 1 SE ($n = 3$). Statistical inference is based on the natural log transformed data. ***$P \leq 0{\cdot}001$, *$P \leq 0{\cdot}05$, †$P \leq 0{\cdot}1$, ns: not significant. DMY: dry matter yield. CAB: change in above-ground biomass under drought relative to control conditions. SR: shallow-rooted, DR: deep-rooted, NL: non-legume, LE: legume.

grasslands at multiple sites. The results highlight: (i) the primary importance of pedo-climatic conditions of the sites for the plants' responses to drought events, (ii) the high resistance of the legumes to severe drought and the great resilience of the non-legumes, resulting in overcompensation of non-legume yields in the post-drought period and coinciding with reduced N limitation of growth, and (iii) the high potential for cropping mixtures to compensate for drought-induced yield losses.

DISTINCTLY DIFFERENT DROUGHT SEVERITY AND PERSISTENCE AMONG SITES

Yields in the rainfed control of all stands at the three sites were comparable (Table 1, Appendix S1); nevertheless, the species responded very differently to the same length of precipitation exclusion at the three sites, as in other multi-site studies (Gilgen & Buchmann 2009; Byrne, Lauenroth & Adler 2013). Species also responded differently during the post-drought period. Site-specific differences in pedo-climatic conditions leading to clearly different severity and persistence of drought stress can explain these results. At Wexford-IE, the very sandy soil (56% sand in top soil) with a low water retention capacity, combined with an unusually low summer precipitation, led to an extreme drought stress with soil matric potentials below −1·5 MPa during the drought treatment at both soil depths (Fig. 1c,f). In contrast, the soils with higher silt and clay content and greater water-holding capacity at both Swiss sites caused soil moisture to reach −1·5 MPa only around halfway through the drought period at 5 cm depth (Fig. 1a,b), even if the absolute amount of excluded precipitation was greater than at the Irish site (Table S2). Due to a lack of precipitation during the post-drought period at the Irish site (Fig. S2), extreme drought stress continued even when the shelters were removed, with values below −1·5 MPa for most of the post-drought observation period; in contrast, adequate water supply was quickly reached at the Swiss sites. Together, this strongly suggests that species were more impaired during and after the sheltered period at the Irish than at the Swiss sites because of site-specific differences in pedo-climatic conditions. Such site-specific differences in soils point to the primary importance of the soil water-holding capacity to buffer increased variability in precipitation due to climate change. Yet, because the extreme drought occurred at only the Irish site, contributions of drought and site-specific differences on the plants' drought response cannot be fully separated. For example, Wexford-IE had the lowest amounts of applied N fertilizers, and plant-available soil N can interact with differential drought stress, as can litter decomposition and soil microbiota, all of which might affect the drought and post-drought responses of grassland species (Bloor & Bardgett 2012).

DROUGHT RESISTANCE DEPENDS ON FORAGE SPECIES, SOIL WATER AND PLANT NITROGEN LIMITATION

Shortage of soil water is generally associated with a shortage in plant-available soil N (Durand, Gonzalez-Dugo & Gastal 2010). We argue that the drought response of our species that differed in rooting depth and N acquisition needs to be viewed in the joint light of both soil water and plant N limitation. The deep-rooted species *C. intybus* was only moderately less impaired by severe drought than the shallow-rooted *L. perenne*, as was the deep-rooted *T. pratense* compared to the shallow-rooted *T. repens* (Fig. 2, compare CAB). This difference was

Table 1. Predicted above-ground biomass yield under rainfed control and drought conditions of monocultures of each of the four species during the second regrowth of a 9-week drought period at three different sites varying in their drought severity, and components of the diversity effect of the equi-proportional four-species mixture (SR: shallow-rooted, DR: deep-rooted, NL: non-legume, LE: legume). For regression estimates, see Table S4

		Monocultures					Equi-proportional mixture						
		Lolium perenne	*Cichorium intybus*	*Trifolium repens*	*Trifolium pratense*	Average monoculture	Yield	Diversity effects†					
		SR-NL	DR-NL	SR-LE	DR-LE			SR*DR		NL*LE		CROSS	
Site	Treatment	(t ha^{-1})¶	(t ha^{-1})	(t ha^{-1})	(t ha^{-1})	(t ha^{-1})	(t ha^{-1})	(t ha^{-1})	(%)‡	(t ha^{-1})	(%)	(t ha^{-1})	(%)
Tänikon-CH	Control	1·51 (0·184)	1·20 (0·190)	1·81 (0·184)	2·84 (0·189)	1·84 (0·114)	2·30 (0·119)	0·01 ns	0	0·22***	12	0·23***	12
(Severe drought stress)	Drought	0·52 (0·185)	0·85 (0·191)	1·30 (0·188)	2·84 (0·188)	1·38 (0·114)	1·84 (0·119)	0·01 ns	1	0·22***	16	0·23***	17
Reckenholz-CH	Control	0·77 (0·213)	2·03 (0·213)	1·27 (0·213)	3·30 (0·213)	1·84 (0·114)	2·60 (0·137)	0·19 ns	10	0·23*	13	0·34**	18
(Severe drought stress)	Drought	0·09 (0·213)	0·98 (0·213)	0·88 (0·213)	2·55 (0·213)	1·13 (0·114)	1·89 (0·137)	0·19 ns	17	0·23*	21	0·34**	30
Wexford-IE	Control	0·79 (0·089)	0·89 (0·089)	1·44 (0·089)	0·99 (0·089)	1·03 (0·051)	1·52 (0·063)	0·11*	10	0·16**	15	0·23***	22
(Extreme drought stress)	Drought	0·13 (0·089)	0·39 (0·089)	0·08 (0·089)	<0·01 (0·089)	0·15 (0·051)	0·19 (0·063)	0·01 ns	10	−0·01 ns	−6	0·04 ns	27

***$P \leq 0{\cdot}001$, **$P \leq 0{\cdot}01$, *$P \leq 0{\cdot}05$, ns: not significant.

[†] The diversity effect is calculated as the difference between the above-ground biomass yield of the equi-proportional four-species mixture and the yield of the average of monocultures and is split into the effects of mixing shallow- and deep-rooted species (SR*DR), mixing non-legumes and legumes (NL*LE), and the crossed effect of mixing a shallow-rooted non-legume with a deep-rooted legume (Lp*Tp) and *vice versa* (Ci*Tr) (CROSS) (eqn 4).

[‡] % change in above-ground biomass yield relative to the yield of the average of monocultures.

[¶] t dry matter yield ha^{-1} (±1 SE).

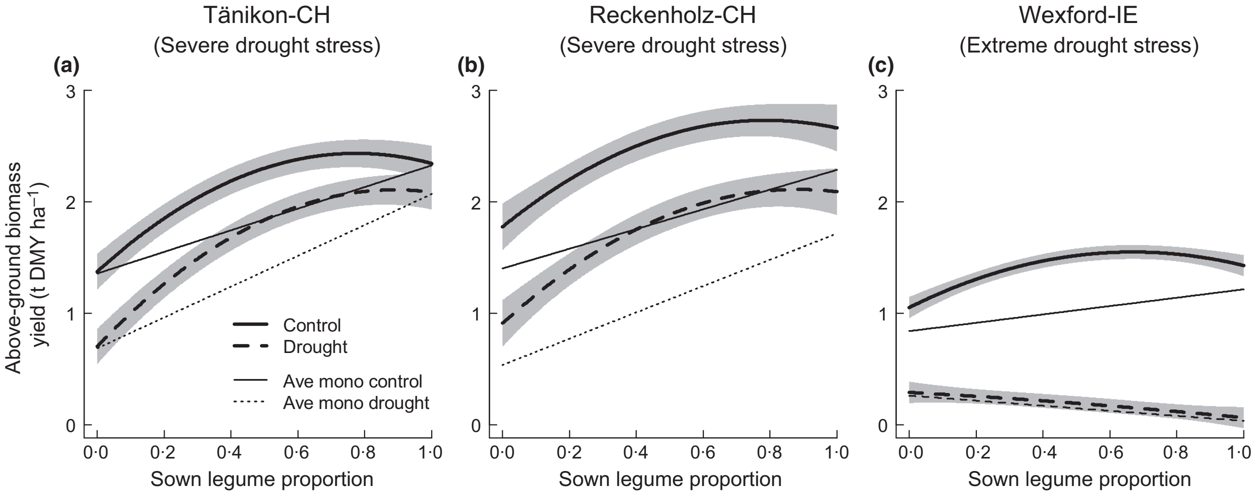

Fig. 4. Predicted above-ground biomass yield of the second regrowth during the drought period for increasing legume proportions under rainfed control and drought conditions at Tänikon-CH (a), Reckenholz-CH (b) and Wexford-IE (c). Predicted lines (± 1 SE, grey shaded) are based on regression analysis (Table 1 and Table S4) and are displayed for mixtures that are equally composed of the two non-legumes and legumes, meaning that the left and right endpoints of lines represent binary mixtures and the prediction at 50% legume proportion represents the equi-proportional mixture (see Table S1 for the design of mixtures). Thin lines represent above-ground biomass yield that could be expected from the weighted average of the respective monocultures (Ave mono) in the absence of any diversity effect. DMY: dry matter yield.

surprisingly small. We infer that more soil water in deeper soil layers might not facilitate drought resistance of deep-rooted species because grassland species under drought do not necessarily shift water uptake towards deeper soil layers (Hoekstra *et al.* 2014; Prechsl *et al.* 2015). Also, shifting resource uptake to deeper layers comes with a trade-off between water and nutrient availability; while more water is generally available in deeper soil layers (Fig. 1 this study; Gonzalez-Dugo *et al.* 2005; Hoekstra *et al.* 2014 for similar systems), nutrient concentration decreases considerably with increasing soil depth (Dolan *et al.* 2006), especially in fertilized agricultural systems. The benefit of accessing water from deeper soil layers may thus be counteracted by lower nutrient availability there. In agreement with a recent study (Hoekstra *et al.* 2015), our results suggest that the 'deep rooting' trait might contribute to drought resistance, but that the effect could be small and might become important only under extreme drought conditions (see Wexford-IE, Fig. 2c).

Nitrogen limitation in both non-legumes was substantial and even increased in *C. intybus* under severe drought (Fig. 5a,b). Under water deficit, Gonzalez-Dugo *et al.* (2012) demonstrated in three forage grass species that N uptake and root-to-shoot N translocation was restricted, which lowered N concentration in above-ground biomass and limited production of total plant biomass. Thus, we suggest that the strong drought impairment in biomass yield of our non-legumes was related not only to an increased water shortage but also to N shortage under drought, which led to an increased N limitation of *C. intybus* (Fig. 5). The lack of such a drought-induced increase in N limitation of *L. perenne* is most probably related to a methodological problem: the CNC is well defined for above-ground biomass yields > 1 t ha^{-1}, but not for yields < 1 t ha^{-1} (Lemaire & Gastal 1997). Because biomass yield of *L. perenne* was in general very low under drought (Table 1: 0·09–0·52 t ha^{-1}), any change in CNC could not be reasonably calculated.

In contrast, both legumes were not N limited, even under drought. This indicates that the good yield performance of the legumes under severe drought was related to their benefit from N_2 fixation which, under drought, made them suffer from water shortage but not from apparent N shortage. It is known from growth chamber experiments that symbiotic N_2 fixation is inhibited under dry conditions (e.g. Serraj & Sinclair 1996). However, the lack of N limitation in the legumes under severe drought strongly indicates that symbiotic N_2 fixation was only downregulated to an extent that was still able to satisfy the N demand of the plant, which, under drought, was smaller due to low availability of water. Such a response was also found in *T. repens* for other growth-limiting resources (e.g. phosphorus; Almeida *et al.* 2000) and corresponds to the concept of N feedback regulation of symbiotic N_2 fixation (Hartwig 1998).

RESILIENCE OCCURRED QUICKLY AND LED TO HIGH YIELDS, RESULTING IN LITTLE CUMULATIVE DROUGHT EFFECT

At adequate water supply (Swiss sites), resilience of formerly drought-stressed stands occurred quickly and completely for all four species. Grasslands of low-to-medium management intensities have been shown to be quite resilient after drought, within the same growing season (Gilgen & Buchmann 2009) or in the subsequent year (Vogel,

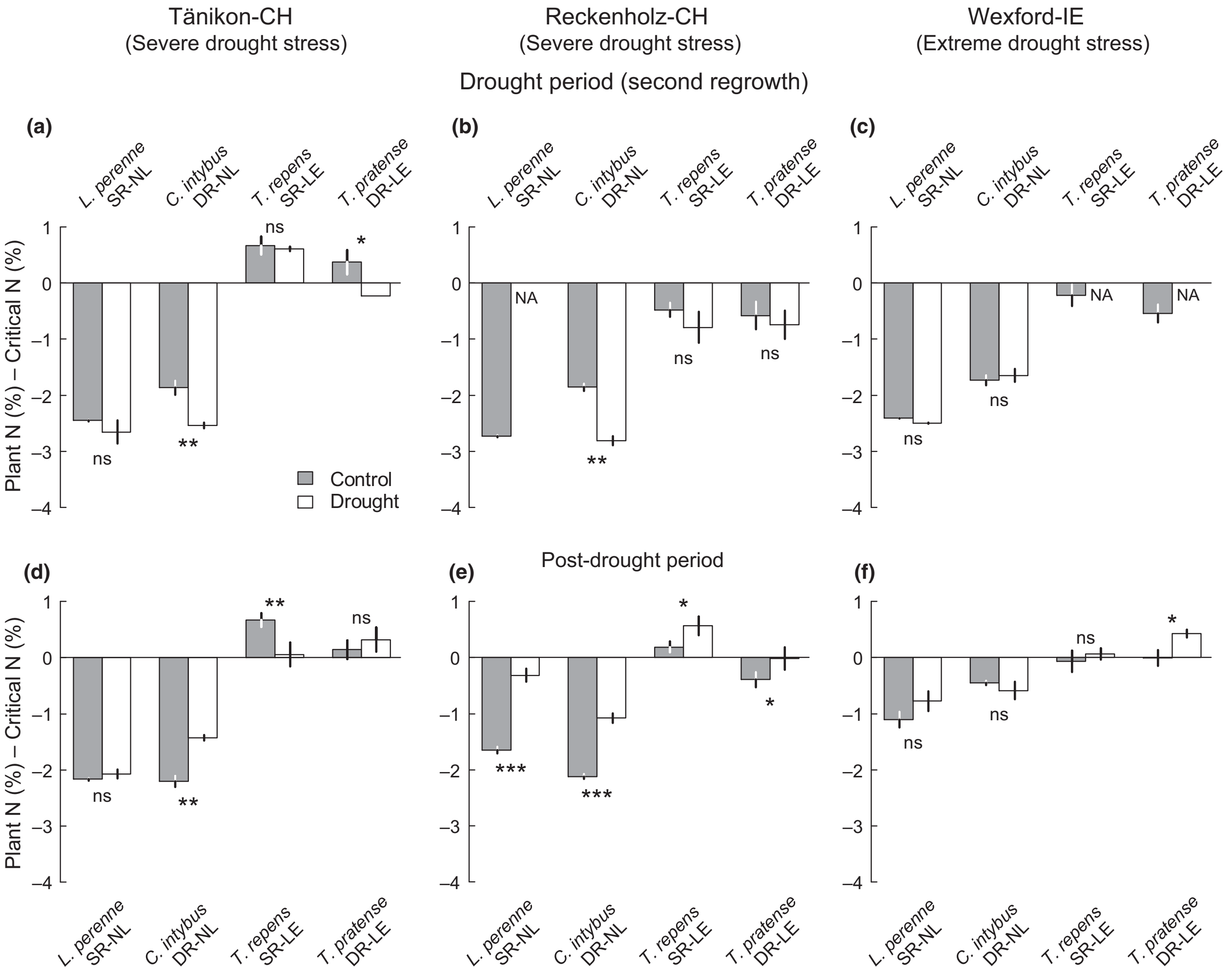

Fig. 5. Difference between above-ground plant N concentration and the critical N concentration (CNC) for N-unlimited growth of the four species in monocultures for the second regrowth during the drought period (a–c) and for the post-drought regrowth (d–f) under rainfed control and drought conditions at Tänikon-CH (a and d), Reckenholz-CH (b and e) and Wexford-IE (c and f). Negative values indicate N limitation. Displayed are means ± 1 SE ($n = 3$); missing SEs are due to unavailable data in replicates. $^{***}P \leq 0{\cdot}001$, $^{**}P \leq 0{\cdot}01$, $^{*}P \leq 0{\cdot}05$, ns: not significant. SR: shallow-rooted, DR: deep-rooted, NL: non-legume, LE: legume. NA: data not available due to very low biomass yield.

Scherer-Lorenzen & Weigelt 2012; Hoover, Knapp & Smith 2014). In our study, the formerly drought-stressed non-legumes even outperformed their control by more than 60%, which was unexpected and has never been reported before. Our N limitation results (Fig. 5) indicate that this was due to clearly reduced N limitation in formerly sheltered plots. We explain this by N fertilizer that was applied in equal quantities to the rainfed and the sheltered plots. This was barely dissolved under the shelters during the drought period and must have been dissolved during the post-drought period, leading to a peak in N availability.

A quick return of the soil water status to non-stressed conditions after the end of the drought period is a crucial requirement to examine plant resilience. This requirement was not fulfilled at Wexford-IE, where a prolonged drought stress occurred with soil moistures below the critical matric potential of −1·5 MPa for more than 4 weeks during the post-drought period (Fig. 1c). Remarkably, the last 2 weeks of the post-drought period, where improved water supply occurred, were sufficient to allow both non-legume species to achieve the same yield in the formerly drought-stressed treatments as in the rainfed control (Fig. 2). This points to the high and fast resilience potential even after an extreme and extended drought stress as at the Irish site. Notably, resilience of yield was not only fast but also persistent as no significant drought impairment was apparent for non-legumes and legumes at all sites at the first harvest of the subsequent year (Appendix S1).

As a result of the species' reactions during and after the drought period, a negative cumulative drought effect was only found in a few species × site combinations (Fig. 3). Species had different strategies to achieve this: they were either remarkably drought resistant (legumes) or, if not resistant, very resilient, even outperforming the rainfed control (non-legumes) (Fig. 2). A recent study, exerting a similar period of simulated drought to less intensively managed natural grassland, found little or no drought effects on yields cumulated over stressed and subsequent post-drought periods (Hartmann & Niklaus 2012). It thus seems that these species associated with intensively managed grassland might be able to cope with predicted drought scenarios, unless the stress becomes extreme; the latter may also be caused by repeatedly occurring droughts.

OVERYIELDING IN MIXTURES COMPENSATED FOR DROUGHT IMPAIRMENT IN SPECIES' MONOCULTURES

Overyielding of mixtures with species differing in their functional traits has recently been demonstrated in intensively managed grassland under ambient climatic conditions (Nyfeler *et al.* 2009; Finn *et al.* 2013). Here, we show that overyielding was also evident under severe drought conditions, especially due to synergistic interactions between non-legume and legume species (Fig. 4a,b, Table 1). Because our results demonstrate that N was a main growth-limiting resource (Fig. 5), we suggest the three most relevant processes underpinning this synergism to be: (i) the access to the unlimited N source of the atmosphere through the legumes (Høgh-Jensen & Schjoerring 1997; Lüscher *et al.* 2014), (ii) an increased capability of non-legumes to acquire N from non-symbiotic sources in the presences of legumes (Nyfeler *et al.* 2011) and (iii) an increased utilization of fertilizer and soil N resources through spatial niche complementarity between shallow- and deep-rooted species (Table 1: SR*DR and CROSS effects) (van Ruijven & Berendse 2005; Mueller *et al.* 2013). The size of overyielding under severe drought was so large that mixtures with approximately equal species proportions at least achieved the yield of the average of the rainfed monocultures (Fig. 4a,b; Table 1). This is a highly relevant result for practical grassland management as it provides evidence that the use of designed mixtures instead of monocultures can be an adaptation measure to compensate for yield losses under predicted drought scenarios.

Although we found only a small or no cumulative drought effect on monocultures' yield, there were very strong short-term detrimental effects of drought on yields of some monocultures, in particular on *L. perenne* (Fig. 2). *L. perenne* is currently the most widely used monoculture crop in intensively managed temperate grasslands; yet, this species is known to perform badly under restricted growth conditions. This suggests that severe forage limitations could occur during drought events. It further highlights the value of forage mixtures because, under severe drought (Swiss sites), mixture yields largely exceeded those of *L. perenne* and even attained yields of the average of rainfed monocultures. The average of monocultures is the most sensible reference here as the identity of the best-performing monoculture can switch across sites, management conditions and over time (Finn *et al.* 2013). Thus, optimizing mixtures through targeted preselection of species with functional traits to maximize niche complementarity is a highly successful strategy to produce overyielding and even transgressive overyielding (mixture performance better than the best monoculture) (Nyfeler *et al.* 2009; Finn *et al.* 2013).

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Data accessibility

Dry matter yield and plant N data are available from Dryad Digital Repository http://dx.doi.org/10.5061/dryad.1f446 (Hofer *et al.* 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Supporting text and information on the methods and analyses.

Table S1. Sowing proportions of the four species in experimental stands.

Table S2. Meteorological data of the three sites and the drought period.

Table S3. Harvest dates during the experimental year.

Table S4. Regression estimates of above-ground biomass yield.

Fig. S1. Rainout shelter and experimental field during the drought period.

Fig. S2. Air temperature and daily precipitation.