biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2024) 68: 60-69 DOI: 10.32615/bp.2024.004

Special Issue "EUCARPIA2023"

Differential biomass and nutrient accumulation in perennial ryegrass accessions under excess water treatment in field conditions during winter

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Abstract

Excess water is an abiotic stress in plants, but the level at which excess water becomes varies widely between plant species. We conducted a two growing season replicated excess flooding experiment that was planted with 24 accessions of perennial ryegrass which had been vegetatively propagated to ensure equal representation of genotypes within an accession, both cultivars and ecotypes, from various geographical origins. The excess water treatment applied over the winter periods was achieved with irrigation. Yields increased in the winter-flooded treatment in contrast to the non-artificial watered control treatment significantly in 2017. In 2018 the same trend could be seen, but was not significant. Differences in composition of macro- and micronutrient profiles were observed. Sulphur was the only element with highly significantly increased concentration (0.25%) in flooded samples compared to control. Phosphorus, copper, iron, manganese, and molybdenum decreased statistically significantly under flooded conditions. In conclusion, perennial ryegrass is coping extremely well with excess water supplied over the winter period and can utilise it effectively in spring.

Keywords: chemical composition, excess water, *Lolium perenne*, macro elements, trace elements, waterlogging.

Introduction

Permanent agricultural grassland and other natural and semi-natural grassland areas in Europe provide a wide range of ecosystem services and are a key component of agroecological systems (Eurostat 2018). Many opportunities can be derived from grass biomass, *e.g.*, source of high quality protein feed, high quality protein food after processing, alternative energy sources from anaerobic digestion, and source of high-value compounds (Shinde *et al.* 2023). However, agricultural grasslands are negatively impacted by increasing incidences of abiotic stresses associated with extreme weather events due to climate change. The abiotic stress conditions that are posing challenges for plants and grasslands include salinity, drought, flooding, cold, and freezing (Loka *et al.*

Received 15 December 2023, *last revision* 9 February 2024, *accepted* 3 April 2024.
Abbreviations: CTL - mean of the control treatment; FL - mean of waterlogging treatment; IPCC - Intergovernmental Panel on Climate Change; LOQ - limit of quantification; RDD - relative differences between sample duplicates.
Acknowledgements: This research was supported by an Irish Department of Agriculture, Food and the Marine (DAFM) Research Stimulus Grant (VICCI - Grant No: 114/S/819 4/S/81) to SB and CKYN. Thank you to Denis Brennan (*Teagasc*) for water nutrient analysis and Jim Grant (*Teagasc*) for feedback on the trial plan and statistics. We also want to recognise the analytical work (ryegrass digestion) carried out by students during their academic internship.
Conflict of interest: The authors declare no conflict of interest.

2019). Climate associated changes in precipitation have led to increased incidences of extreme events like flooding (IPCC 2014). Extreme flood events may favour ruderal plant species which are able to respond rapidly to environmental change. Additionally, wet grasslands may dry out during heatwaves and drought. As such, forage for livestock will likely become less reliable, which necessitates adaptations to cutting and grazing regimes (Joyce *et al.* 2016). This can be further exacerbated by challenges to machine operations in wet environments that can result in substantial damage to soil, grasslands, and ultimately productivity. Additionally, it may not be possible to operate machinery during particularly wet periods (Hargreaves *et al.* 2019).

Under flooding or waterlogging only the root sphere is fully exposed to water. In contrast, the shoots are either partly or completely covered in water under submergence. Oversaturation of the soil can impact on the whole ecosystem when all available oxygen in the soil is rapidly consumed by soil microbial organisms and by plant root respiration (Vashisht *et al.* 2011). Some plant species can overcome the consequences of excess water by forming aerenchyma, an anatomical adaptation to facilitate exchange of gases between the shoot and root, however perennial ryegrass has not been noted for this coping mechanism.

Partial or full submergence enhance stress further by the attenuation of carbon dioxide influx for photosynthesis. Plants have evolved an escape mechanism underpinned by induction of shoot elongation to enable the shoot to emerge from submergence. The extra growth requires additional oxygen regulated by the hormone ethylene (Voesenek *et al.* 2003).

Species- and genotype-dependent variation in adaptation to flooding stress has been found in several perennial grass species. McDonald *et al.* (2002) characterised *Phalaris arundinacea* and *Arundo donax* as well-adapted to waterlogging conditions. Within species differences in waterlogging were found in *Dactylis glomerata* (Etherington 1984). For some species, *e.g.*, *Panicum virgatum*, their habitat (lowland *vs*. upland) have also been shown to contribute to flooding stress tolerance (Barney *et al.* 2009). Using a panel of 100 perennial ryegrass accessions, Yu *et al.* (2012) showed differential responses to seven days of submergence and seven days of recovery, suggesting a potential for breeding for flooding tolerance. The genetic potential for breeding for flooding tolerance has also been demonstrated in a *Festulolium* hybrid between *Lolium perenne* and *Festuca pratensis* that has been shown to be able to reduce the runoff by 51% compared to a leading UK nationally recommended *Lolium perenne* cultivar over two years. This reduced runoff was due to intense initial root growth followed by rapid senescence, especially at depth (Macleod *et al.* 2013).

Waterlogging affects plant nutrients due to alterations in element solubility in the soil when it is under anoxic conditions. Floods are often accompanied by a decrease in soil pH, which increases the solubility of macro and micronutrients, including iron, manganese, and phosphorus as well as potential toxic metals. A strong reduction in diffusion of gases in floodwaters limits the availability of oxygen and carbon dioxide for aerobic respiration and photosynthesis and adds on to nutrient imbalances (Bailey-Serres *et al.* 2012). In an experiment with eight grasses and four legume species under flooding conditions, it was demonstrated that primary production was negatively impacted, while nitrogen losses in the form of the potent greenhouse gas, nitrous oxide, were enhanced. Interestingly, the grasses were comparably better resistant to flooding compared to the legumes, whereas the legumes exhibited better recovery overall (Oram *et al.* 2021). Although it is believed that productivity of grasslands is lower on marginal land sites, including very wet sites, it has been reported that under wet and flooded sites, comparably high yields can be achieved (Meehan *et al.* 2017).

The aim of this study is to explore the tolerance potential of perennial ryegrass accession to winter flooding by determining biomass production and elemental composition in the subsequent spring vegetation period.

Materials and methods

Plants and cultivation: Seeds of 24 perennial ryegrass cultivars and ecotypes were received from various breeders. Eight single plants per accession were raised in small insert trays in a glasshouse in Oak Park Carlow/Ireland. The seedlings were tillered in the glasshouse into multiple identical plants and were allowed to grow to a multi tiller stage until transplanted as mini swards consisting of four clonal plants of one genotype in May 2016 into the field in Oak Park Carlow (52°51'43"N, 6°55'07"W; 58 m above sea level). The plants were cut at regular intervals during the establishment year to allow them to grow into dense mini lawns plots (35 × 35 cm which corresponds to 0.1225 m^2).

The field design was a randomized complete block design. The experiment had 192 entries (24 accessions with eight individuals each), one flooded and one control treatment and each treatment consisted of three blocks.

Due to the small size of the plots manual harvests corresponding to the first cut of the year were carried out in 2017 and 2018 (2017: 03/05/ and 04/05/; 2018: 30/04/ and 01/05/). These harvest dates are typical first cut dates in Ireland. Yield was measured as fresh mass (FM), by weighing the biomass harvested in 0.1225 m^2, and as dry mass (DM), by weighing the same biomass after drying at 60°C until constant mass was attained (*ca.* 48 h). The remainder of each of those two years the plots were cut for maintenance without measuring yield.

Flooding treatment: The flooding treatment plots were artificially waterlogged on the 5th of December 2016 using a boom irrigator due to the dry conditions in this particular winter. The control treatment plots did not receive any artificial water addition. Throughout three months, a total of 1 620 mm of water was applied evenly over the waterlogged treatment blocks to achieve artificial waterlogging. In the winter field season 2017/2018 we relied on natural flooding and only added 36 mm of

The twenty-four accessions of perennial ryegrass in alphabetical order, their origin, ploidy status, maturity group, and further information. *These breeders' seeds were received coded, but destination of breeding market is known.

Accession	Breeder	Ploidy	Maturity group	Recommended list
AberGreen	IBERS	2n	intermediate	UK
AberZeus	IBERS	2n	intermediate	UK
Arolus	Agroscope	2n	early	Switzerland
Ba14155	IBERS	2n	intermediate	UK
BAR03*	Barenbrug	2n	intermediate	Romania
BAR05*	Barenbrug	2n	intermediate	Netherlands
BAR06*	Barenbrug	2n	late	Netherlands
Carraig	Teagasc	4n	intermediate	Ireland
Cashel	Teagasc	2n	intermediate	Ireland
Denver	Advanta/DLF	2n	late	Ireland
Giant	Teagasc	4n	intermediate	Ireland
Glencar	Teagasc	4n	late	Ireland
LP0515	Agroscope	2n	early	highland × low land ecotypes
LP1005	Agroscope	2n	early	highland × low land ecotypes
LP9155 (Canis)	Agroscope	2n	intermediate	Released for German market
Picadilly	EuroGrass	2n	late	Ireland
RHZ110123 Ottoberg	Swiss ecotype	2n	early	low land ecotype
RHZ110124 Mümliswil Passwang	Swiss ecotype	2n	early	high land ecotype
RHZ110125 Wildberg	Swiss ecotype	2n	early	low land ecotype
RHZ110127 Bütschwil Zwiselen	Swiss ecotype	2n	early	low land ecotype
Rodrigo	EuroGrass	2n	intermediate	Ireland
Solomon	Teagasc	2n	intermediate	Ireland
Soriento	EuroGrass	2n	late	Ireland
Twymax	CPB Twy./DLF	4n	late	Ireland

Weather data for two seasons of flooding field trial. Data obtained from *Met Éireann* (*Met Éireann* 2019) weather station at Oak Park, Carlow, Ireland. In addition to rainfall, water was added with a boom irrigator (*these values are for the waterlogged treatment only, the control treatment received no additional water besides rainfall).

Season/Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
2016-2017								
Rainfall [mm]	32.3	26.3	80.2	26.2	57.8	66.6	15.8	305.2
Evaporation [mm]	36.5	13.9	10.9	14.9	25.4	51.8	71.2	224.6
Days flooded*	3	3	15	9	13	8	0	51
Added artificial flooding [mm]*	0	0	540	540	540	0	0	1 620
2017-2018								
Rainfall [mm]	62.9	45.8	84.2	108.1	38.7	98.1	73	510.8
Evaporation [mm]	35.6	13	11.9	17.5	24	43.8	77.3	223.1
Days flooded*	4	12	19	17	10	15	8	85
Added artificial flooding [mm]*	36	0	0	0	0	0	0	36

additional water with a boom irrigator over a month to the flooding treatment blocks. The control treatment was slightly elevated and was at no time in both winter waterlogged. Water saturation of the soil in both treatments was recorded with probes (*Delta-T* devices).

Chemical analysis: Water and soil analysis were performed at *Teagasc Johnstown Castle* research laboratory. Briefly, the water samples were acidified (pH < 2) with HCl and stored at 4°C. Total phosphorus (TP) was determined using the *Hach Ganimede* (Düsseldorf, Germany) P analyser, by reaction of phosphate ions with molybdate and antimony ions to form an antimony-phosphomolybdate complex that was reduced to phosphorus molybdenum blue using ascorbic acid at 150°C and 0.6 MPa. The absorbance of this compound was measured spectrophotometrically at 880 nm. Total nitrogen (TN) in the water was determined using the *Hach Ganimede* N analyser, through the oxidation of inorganic and organic bound nitrogen to nitrate with peroxidisulfate using alkaline digestion at 150°C and 0.8 MPa. The nitrate concentration was measured photometrically using UV self-absorption in a differential measurement at 210 and 228 nm. Representative soil samples, collected from the field in October 2015, were dried (40°C), sieved through a 2-mm sieve and homogenized. Soil-available P and K were determined by inductively coupled plasma optical emission spectroscopy (*ICP-OES*) (*5100 ICP-OES*, *Agilent*, Santa Clara, California, USA), after extraction with Morgan's reagent. Moisture and organic matter content of soil were determined gravimetrically, by oven-drying (*Universal Oven UF110*, Memmert, Ireland) the sample at 105°C overnight and subsequently igniting in a muffle furnace (*B180 Muffle Furnace*, Nabertherm, Ireland) at 500°C until constant mass was attained. Soil pH was measured potentiometrically in a 1:5 soil:water with an *InLab®Routine Pro-ISM* combined pH electrode (*Mettle*, Toledo, Spain).

Elemental composition of ryegrass was determined for samples from the 2017 season. An in-house protocol based on *Method 3051A* (Link *et al.* 1998) was used to decompose the plant matrix. Briefly, a 0.50 (± 0.05) g ryegrass sub-sample, previously dried and ground to pass through a 1.0-mm screen using a *Retsch* cutting mill, was accurately weighed into a pre-cleaned digestion liner and decomposed by microwave assisted acid digestion (*Multiwave 3000*, *Anton Paar*, Graz, Austria) using 10 ml of concentrated nitric acid (HNO_3 67%, trace element grade, VWR). The digested sample was poured into a volumetric flask (50 ml), which was subsequently filled to the mark with ultrapure water (conductivity ≤ 0.055 µS cm^{-1}) and the resulting suspension filtered (*MN 640W*, *Macherey-Nagel*, Düren, Germany) to a plastic container. The elemental concentration of Al, Ca, Cu, Fe, K, Mg, Mn, Mo, P, S, and Zn in the digest was determined by *5100 ICP-OES*, *Agilent* (Table 1 Suppl.). Multi-elemental calibration standard solutions were prepared from custom-made commercial multi-element standard solutions (Custom-made multi-element MW digestion calibration stock standard 1 and stock standard 2, *Reagecon*). An independent custom-made commercial standard solution (Custom-made Multi-element MW digestion QC stock standard, *Tellab*) was used to prepare calibration control verification solutions, measured after the calibration standards and at regular intervals during the analysis run to confirm the concentration of the calibration standards and to monitor instrumental drift. To ascertain analytical trueness two control materials (CM) were used: *WEPAL 234.1* [Banana (leaf)/*Musa* sp., Ecuador] and 203.3 [Cabbage (leaf + stalk)/*Brassica oleracea*, Netherlands]. Recoveries in the range of 80 - 120% were obtained. Relative differences between sample duplicates, RDD [%], were used to monitor the precision of the analytical procedure. Sample duplicates were included in each batch. The RDD values were typically ≤ 20%, which was considered acceptable for the methodology used. To assess contamination, reagent blanks (RB) were included in each sample batch.

Statistical analysis: Descriptive analysis, outliers' evaluation, normality, homogeneity of variances, and *ANOVA* were performed using the software *GenStat*, 22nd edition (*VSN International*, Hemel Hempstead, UK). At the population level, analysis of variance was done by entering the values of the eight genotypes per accession as replicates within blocks, *i.e.*, a total of 24 entries per accession per water treatment. The block structure was included and accession, water regime (control *vs*. flooded) and year, were entered as factors. Fresh and dry masses were the variates considered. At the genotype level, the analysis of variance was done using genotype, water regime, and year as factors, a randomized block structure with three replicates per genotype, *i.e*., the accession of each genotype was not taken into account.

Results

Average yields: Fresh yield averages in 2017 and 2018 in the control treatment were 97.1 and 118.8 g, respectively, and in the water excess treatment in 2017 and 2018 137.6 and 180.0 g, respectively.

Dry yield averages in 2017 and 2018 in the control treatment were 33.36 and 36.45 g, respectively, and in the water excess treatment in 2017 and 2018 40.58 and 37.49 g, respectively. In term of dry biomass yield in 2017 under control conditions the top two performers were Ba14155 and LP1955 (43.0 g), followed by Ottoberg (39.6 g) (Table 1). In 2017 under the flooded treatment the top performer was Ba14155 (57.7 g), followed by LP0515 (56.8 g), Solomon (47.7 g), and LP9155 (47.2 g). In 2018 the top performers for dry biomass yield under control conditions were Solomon (46.9 g), Buetschwil (44.5 g), Arolus (44.2 g), and Twymax (41.0 g). In 2018 under the flooded treatment the top performer was Solomon (67.3 g), followed by Rodrigo (59.1 g), Twymax (54.4 g), and Arolus (53.6 g) (Table 1). In general, top performers were breeding materials, but also some ecotypes featured in the top performers like Ottoberg and Buetschwil. One accession bred from ecotypes, LP0515, was also amongst the top performers in 2017 under flooded conditions.

Performance of accessions by year under control and flooded conditions: In 2017 and 2018 accessions which performed better under flooding compared to control conditions were matching between the two years (AberZeus, Arolus, Buetschwil, Carraig, Denver, Giant, LP0515, LP1005, Ottoberg, Rodrigo, Solomon, Twymax, and Wildberg). The comparative top performers under flooding for those two years were Arolus and Solomon (Table 1).

Within accession performance under control and flooded conditions averaged over two years: In 12 out of 24 accessions a high frequency of individuals (≥ 6/8) with higher dry biomass under flooding conditions as compared to control conditions was found: AberZeus (6/8), Arolus (7/8), Ba14155 (7/8), Buetschwil (6/8), LP0515 (7/8), Rodrigo (7/8), Solomon (6/8), Twymax (6/8), Wildberg (7/8), Ottoberg (8/8), LP1005 (8/8), Denver (8/8) (Fig. 1).

Table 1. Average fresh and dry masses across eight genotypes per accession in two harvest years (2017 and 2018) in 24 accessions under control (CTL - mean of the control treatment) and flooding, FL - mean of waterlogging treatment) and minimum (MIN - minimum average value of a genotype) and maximum (MAX - maximum average value of a genotype) values per accession.

Population	Fresh mass [g]												Dry mass [g]											
	2017						2018						2017						2018					
	CTL	MIN	MAX	FL	MIN	MAX	CTL	MIN	MAX	FL	MIN	MAX	CTL	MIN	MAX	FL	MIN	MAX	CTL	MIN	MAX	FL	MIN	MAX
AberGreen	89.1	36.7	247.2	101.0	36.2	175.0	102.9	50.0	142.0	123.4	51.6	244.9	29.2	14.6	65.7	32.6	14.9	48.3	30.6	15.1	41.9	28.3	14.4	51.0
AberZeus	98.4	30.6	146.7	145.6	38.9	208.5	77.1	50.5	110.0	153.4	60.3	301.3	34.4	15.0	46.4	42.8	13.1	57.8	24.7	19.6	31.5	33.0	14.5	63.1
Arolus	119.0	53.6	210.6	155.1	69.9	260.2	154.3	68.9	269.6	241.2	118.8	371.9	38.7	22.0	61.2	48.8	27.1	77.2	44.2	20.7	78.5	53.6	27.1	87.5
Ba14155	137.5	65.2	216.7	204.9	114.8	257.4	112.5	73.5	181.6	151.7	25.4	221.6	43.0	24.7	63.6	57.7	36.4	71.9	33.6	22.9	50.4	34.3	8.6	50.1
BAR03	77.7	23.9	169.5	133.2	15.1	243.8	111.3	65.0	163.8	163.9	82.7	392.1	27.9	12.7	48.1	37.0	6.9	57.5	32.9	19.9	48.9	33.6	14.5	78.8
BAR05	94.1	61.7	132.4	154.2	83.3	201.8	97.2	57.5	156.3	138.2	39.6	231.4	31.1	23.3	40.4	42.1	24.4	55.9	29.5	18.8	46.8	32.6	16.9	48.5
BAR06	84.9	46.5	114.7	114.8	74.7	155.2	156.2	68.9	229.1	198.6	89.3	358.7	28.2	17.7	36.3	34.1	24.1	44.3	44.5	22.6	61.4	44.1	21.0	80.6
Bütschwil	68.5	39.7	94.0	121.1	88.7	221.5	95.2	39.6	167.2	146.1	91.7	205.0	24.0	15.9	31.2	38.5	29.5	63.2	28.2	10.7	48.7	33.0	22.4	44.1
Carraig	83.9	51.5	167.8	103.7	76.0	206.0	112.1	54.0	219.8	181.4	78.3	399.5	26.4	19.1	48.1	30.2	22.8	54.0	32.9	18.3	62.3	41.9	17.3	89.0
Cashel	101.2	76.2	167.2	125.8	80.8	163.2	111.1	75.1	152.7	141.8	29.2	203.3	32.1	24.2	49.3	38.1	26.3	46.9	32.9	20.4	43.1	32.4	7.3	39.9
Denver	94.3	33.5	182.2	129.3	72.7	184.0	125.2	85.8	183.1	193.0	118.8	249.4	30.0	15.2	52.9	35.6	22.2	50.4	34.6	26.2	45.6	43.1	28.5	56.5
Giant	119.4	36.2	218.7	153.0	41.1	368.8	134.3	78.3	247.1	216.8	140.6	487.9	35.5	14.5	79.8	41.7	16.3	91.6	39.1	23.8	63.1	45.9	30.9	96.7
Glencar	68.6	22.8	126.9	80.8	41.1	134.0	99.3	64.0	147.3	129.9	36.2	236.3	24.9	13.3	38.1	26.6	15.6	43.9	29.3	17.9	44.8	29.5	7.3	53.1
LP0515	108.0	40.1	176.6	187.0	87.5	298.5	121.4	68.8	202.1	186.8	73.3	336.2	36.5	17.2	58.0	56.8	29.4	88.7	35.8	22.6	58.0	43.0	19.1	80.4
LP1005	85.3	38.2	171.2	151.7	79.9	251.8	83.4	60.5	144.1	149.6	73.6	328.7	29.8	16.6	50.3	46.2	29.2	75.8	26.4	19.3	44.2	34.0	17.0	73.3
LP9155	138.7	86.3	227.2	159.9	40.0	223.0	120.6	74.2	164.6	159.7	44.0	284.2	43.0	28.5	57.0	47.2	15.6	63.0	36.2	23.6	51.4	36.3	10.8	66.1
Mümliswil	75.4	20.7	166.4	119.1	39.0	203.6	113.5	72.8	138.3	146.3	83.1	216.4	27.0	10.8	52.1	35.8	16.0	58.2	32.0	22.4	37.7	33.2	20.1	48.4
Ottoberg	107.9	60.3	157.8	167.9	95.1	230.4	121.3	66.2	171.0	183.2	105.3	246.9	39.6	24.7	51.7	55.4	37.6	71.3	36.6	20.7	50.7	41.1	24.7	51.6
Picadilly	69.1	38.5	141.7	106.7	74.1	158.8	131.0	78.7	190.3	166.0	52.7	296.9	25.8	18.1	42.5	33.5	24.7	46.9	39.3	26.3	54.3	37.5	13.0	63.5
Rodrigo	111.3	61.2	166.7	147.6	74.1	158.8	138.7	88.9	192.6	262.1	52.7	296.9	33.8	22.6	48.4	41.5	30.2	55.6	40.0	25.7	51.2	59.1	37.4	80.2
Solomon	119.0	50.8	223.7	161.8	43.7	338.6	173.0	88.4	399.2	303.2	93.8	590.8	36.9	18.5	59.7	47.7	17.4	86.9	46.9	23.1	99.7	67.3	21.7	124.6
Soriento	95.4	60.4	147.7	110.6	67.4	191.8	124.3	83.0	191.7	178.4	72.1	297.6	30.4	22.1	44.2	32.0	21.4	50.6	36.6	26.6	54.2	39.6	18.2	63.6
Twymax	105.8	41.7	171.4	144.6	77.8	233.7	152.9	110.1	209.2	259.2	98.8	421.6	32.1	17.9	43.9	39.0	24.6	60.2	41.0	16.1	55.9	54.4	23.6	80.7
Wildberg	78.9	40.5	122.5	123.8	66.0	192.2	82.2	38.7	146.0	161.4	106.9	207.9	28.9	17.6	43.5	39.3	22.6	55.9	24.4	14.3	43.2	37.0	19.5	52.0

Analysis of variance (*ANOVA*) at the accession level (Table 2 Suppl.) for both fresh and dry mass had significant or highly significant variation amongst accessions and water treatments. Year had a significant impact only in fresh mass production, with increased biomass observed in 2018. Significant 2-way interaction was observed for accession × year and water treatment × year, but not for accession × water treatment, for both fresh and dry mass. *ANOVA* at the genotype level (Table 3 Suppl.) for both fresh and dry mass had significant or highly significant variation amongst genotypes and water treatments. As verified for accessions, year variation was highly significant for fresh mass, but not for dry mass. At the genotype level, all three 2-way factor' interactions (genotype × year, water treatment × year, and genotype × water treatment) were statistically significant to explain variation in fresh and dry mass. No significant differences in fresh and dry mass were identified for 3-way interactions of factors at accession or genotype levels.

Water and soil chemical characterization: The nutrient input from lake water in 2017 were 1 kg P ha^{-1} and 10 kg N ha^{-1}. In 2018 very little artificial irrigation was added to the trial and hence the input from lake water was negligible. Chemical analysis before water treatment, characterized the field as having a neutral mineral soil with adequate nutrients' level for grassland, according to national indices (Wall and Plunkett 2020), with no inputs required. Accordingly, soil pH was 7.3, soil organic matter was 5.1%, available P and K were 7.2 and 125 mg L^{-1}.

Average content of elements in ryegrass: From the macronutrients measured in ryegrass samples (control treatment), K showed the highest content (2.0%), followed by Ca (0.55%), P (0.25%) ~ S (0.23%), and Mg (0.10%). Micronutrients content in control ryegrass samples was as follows: Fe (73 mg kg^{-1}) > Zn (22 mg kg^{-1}) > Mn (16 mg kg^{-1}) > Cu (6.2 mg kg^{-1}) > Mo (3.5 mg kg^{-1}) (Fig. 1 Suppl.). The overall mean content of Al in control samples was 31 mg kg^{-1}. While the order presented above was also observed for the flooded ryegrass samples, the mean values for some elements differed: S was the only element with increased concentration (0.25%) in flooded samples compared to control ($P < 0.001$), while P decreased slightly to 0.23%, albeit significantly ($P < 0.001$); Cu concentration decreased to 5.6 mg kg^{-1} (*ca.* 10% lower, $P < 0.001$), Fe and Mn concentrations decreased to 59 and 13 mg kg^{-1}, respectively (*ca.* 20% lower, $P < 0.001$), while Mo concentration decreased the most to 1.4 mg kg^{-1} (*ca.* 60% lower, $P < 0.001$). The overall mean concentration of Al in flooded samples was 21 mg kg^{-1}.

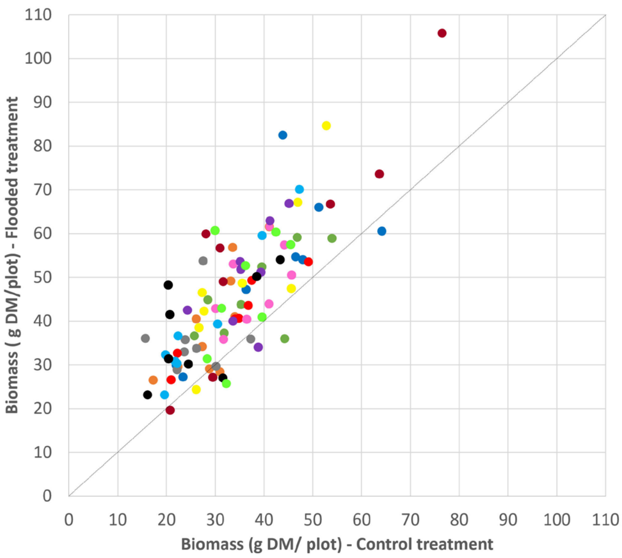

Fig. 1. Biomass of individual genotypes within populations in control *vs*. flooded treatments. Colour of points represented population: AberZeus *orange*, Arolus *dark blue*, Ba14155 *dark green*, Bütschwil *grey*, Denver *red*, LP0515 *yellow*, LP1005 *light blue*, Rodrigo *violet*, Solomon *brown*, Twymax *light green*, Wildberg *black*. The line represents the 1:1 ratio.

Elemental concentration of accessions under control and flooded conditions: The average elemental content for each accession and water treatment is presented in Table 2. Analysis of variance revealed that the content varied significantly between accessions ($P < 0.001$) and between water treatments ($P < 0.05$) for all elements evaluated. Compared to the overall means, accessions Denver, Soriento, and Twymax showed consistently high elemental content while Ba14155, Bar08, and Ottoberg presented lower content for most of the elements. Three distinct trends were observed for the variation in elemental content of the 24 accessions with water treatment (control/flooded): for Ca, K, Mg, and Zn, the mean content of control and flooded samples was essentially the same, although one to three accessions revealed small, but significant differences; for S, the mean content of flooded samples was significantly higher than in the control samples for all accessions, except for Solomon (equal content for control/flooded treatments); for the remaining elements (Al, Fe, P, Mn, Mo, and Cu), the mean content in samples from the flooded treatment was lower than in the control samples for all the accessions, and that difference was significant for most of the accessions (1 to 7 accessions showing no significant difference).

Within accession elemental content under control and flooded conditions: In the nested *ANOVA* to accommodate variation within accessions the elemental content had significant ($P < 0.05$) or highly significant ($P < 0.001$) variation with all genotypes within accession, and most genotypes within accession by treatment (exception for K and Cu). Genotypic variation is presented for accessions Denver and Ba14155 in relative difference between control and flooded for K, P, S, and Mo content (Fig. 2). These were chosen as examples with high and lower elemental content, respectively.

Discussion

The experiment was conducted with flooding and waterlogging over the winter months over two winter

Table 2. Average concentration of Al, Ca, Cu, Fe, K, Mg, Mn, Mo, P, S, and Zn in ryegrass accessions (DM) for control and flooded treatments. Descriptive statistics is also included for each element for control (CTL) and flooded (FL) samples.

Accessions	Al [mg kg^{-1}]		Ca [%]		Cu [mg kg^{-1}]		Fe [mg kg^{-1}]		K [%]		Mg [%]		Mn [mg kg^{-1}]		Mo [mg kg^{-1}]		P [%]		S [%]		Zn [mg kg^{-1}]	
	CTL	FL	CTL	FL	CTL	FL	CTL	FL	CTL	FL	CTL	FL	CTL	FL	CTL	FL	CTL	FL	CTL	FL	CTL	FL
AberGreen	29	20	0.53	0.70	5.7	5.1	72	59	1.80	1.69	0.084	0.086	13.7	12.5	2.47	1.25	0.207	0.175	0.224	0.234	17.0	16.5
AberZeus	23	19	0.71	0.60	5.8	5.0	69	58	1.89	1.84	0.093	0.095	14.8	13.6	3.43	1.31	0.221	0.194	0.213	0.224	18.4	16.7
Arolus	24	20	0.49	0.31	5.1	4.6	57	52	2.06	1.85	0.111	0.116	11.8	9.4	2.78	1.23	0.257	0.225	0.211	0.229	22.3	22.5
Ba14155	27	20	0.58	0.77	5.1	4.3	56	49	1.98	1.89	0.088	0.090	13.6	10.1	3.12	1.10	0.209	0.179	0.193	0.209	16.5	14.7
Bar03	27	22	0.67	0.52	6.8	6.4	68	61	2.05	2.14	0.098	0.109	15.5	13.0	3.41	1.79	0.246	0.235	0.243	0.273	21.6	22.3
Bar05	32	19	0.69	0.53	7.7	6.3	81	62	2.33	2.25	0.101	0.105	18.1	15.0	4.08	1.97	0.283	0.250	0.266	0.275	24.6	24.4
Bar06	35	18	0.59	0.52	8.0	6.9	91	63	2.18	2.12	0.108	0.105	20.4	16.3	4.15	1.95	0.256	0.245	0.244	0.273	23.7	22.1
Butschwil	35	23	0.44	0.41	5.7	5.5	73	67	1.96	2.04	0.098	0.105	16.1	13.1	3.08	1.24	0.247	0.239	0.217	0.243	24.7	24.0
Carraig	37	25	0.54	0.61	6.6	5.7	82	63	2.13	2.02	0.095	0.093	15.5	11.2	3.47	1.52	0.246	0.232	0.227	0.244	21.7	19.5
Cashel	38	25	0.49	0.37	6.2	5.5	88	68	2.25	2.13	0.102	0.105	17.0	12.3	2.95	1.21	0.249	0.226	0.243	0.261	23.1	21.5
Denver	39	29	0.68	0.64	7.2	6.9	93	75	2.21	2.30	0.118	0.125	18.0	15.8	4.36	2.05	0.274	0.263	0.246	0.284	26.6	26.4
Glencar	44	26	0.48	0.47	6.9	6.0	94	67	1.97	1.78	0.087	0.094	16.4	12.6	4.16	1.42	0.271	0.233	0.232	0.275	20.6	17.8
LP 1005	26	20	0.48	0.47	5.7	5.3	62	53	1.85	1.74	0.100	0.106	16.0	11.6	2.74	1.16	0.237	0.223	0.204	0.208	23.4	25.2
LP 9155	25	17	0.58	0.39	6.0	5.1	71	54	2.11	1.85	0.114	0.115	16.5	11.6	3.49	1.30	0.275	0.231	0.230	0.239	22.9	19.5
LP0515	27	20	0.51	0.48	5.7	5.1	63	55	1.82	1.66	0.109	0.109	15.9	13.6	3.13	1.36	0.241	0.217	0.214	0.215	25.8	24.5
Mumliswil	27	22	0.51	0.44	5.3	5.0	62	53	2.01	1.95	0.105	0.111	18.3	12.5	3.70	1.28	0.277	0.242	0.228	0.244	22.7	23.1
Ottoberg	23	22	0.37	0.45	5.5	5.0	55	55	1.63	1.67	0.094	0.098	16.5	12.9	3.18	1.12	0.222	0.204	0.185	0.220	24.6	25.5
Picadilly	36	23	0.62	0.55	6.7	6.1	77	64	1.96	1.95	0.096	0.096	17.0	14.2	3.25	1.62	0.235	0.214	0.239	0.262	21.5	21.0
Rodrigo	32	22	0.57	0.86	7.0	6.2	79	64	2.20	2.17	0.112	0.108	16.9	14.0	3.95	1.67	0.259	0.235	0.240	0.276	23.8	20.8
Solomon	20	16	0.49	0.55	5.7	5.2	67	49	1.97	1.93	0.097	0.100	17.1	12.4	3.09	1.38	0.215	0.208	0.215	0.215	18.3	17.8
Soriento	46	22	0.82	0.54	7.3	6.4	95	67	2.26	2.18	0.118	0.120	18.9	14.7	4.20	1.56	0.271	0.246	0.267	0.283	24.9	23.3
Twymax	38	23	0.55	0.63	7.7	6.7	95	68	2.42	2.29	0.107	0.105	16.8	13.3	4.14	1.53	0.272	0.233	0.256	0.280	23.0	22.1
Descriptive statistics (all values)																						
Mean	31	21	0.55	0.54	6.2	5.6	73	59	2.04	1.97	0.101	0.103	16.1	12.8	3.47	1.43	0.249	0.225	0.226	0.246	22.3	21.2
Median	27	20	0.54	0.53	6.0	5.5	69	57	2.01	1.95	0.101	0.101	15.1	12.6	3.43	1.32	0.251	0.224	0.219	0.243	21.9	20.8
Min	7	6	0.25	0.31	3.2	3.1	27	29	1.31	1.28	0.058	0.059	6.3	6.3	0.71	0.39	0.137	0.110	0.132	0.140	10.8	8.6
Max	76	53	1.16	1.07	9.6	9.6	146	123	2.99	2.88	0.166	0.163	34.9	24.7	7.00	3.88	0.359	0.345	0.354	0.386	43.8	42.9
1st quartile	20	15	0.45	0.45	5.3	4.8	56	47	1.81	1.74	0.090	0.090	12.4	10.8	2.69	1.02	0.226	0.201	0.195	0.215	18.7	17.5
4th quartile	39	26	0.64	0.61	7.1	6.3	86	67	2.25	2.21	0.111	0.115	18.8	14.7	4.17	1.71	0.272	0.250	0.256	0.271	25.5	24.2

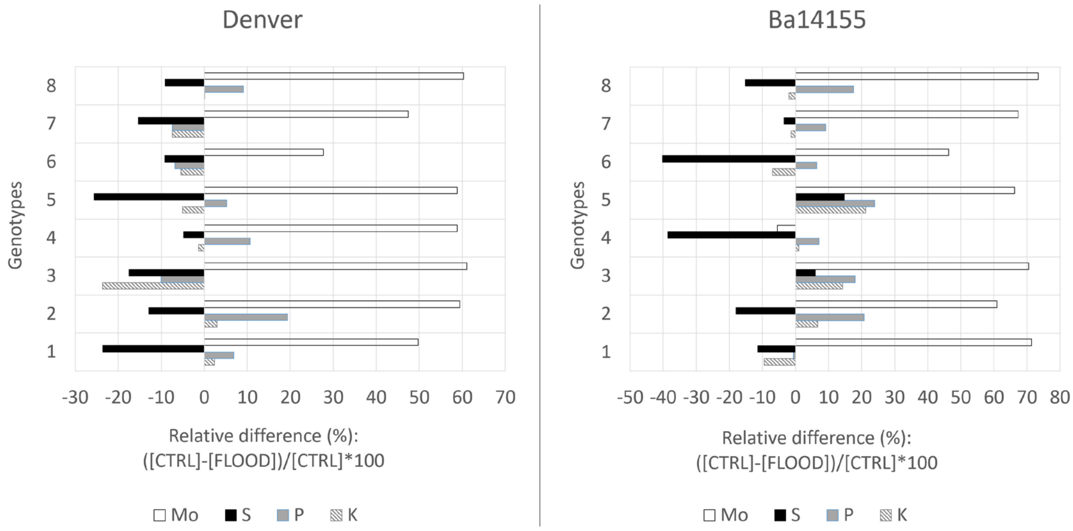

Fig. 2. Genotypic variation in K, P, S, and Mo in accessions Denver and Ba14155 relative to water treatment. Variation within genotype is presented as the relative difference between elemental content in control and flooded treatments.

periods, but the trial was never submerged. The experiment of Yu *et al.* (2012) was a submergence trial; hence our experiment is not fully comparable to the trial reported by Yu *et al.* (2012). But we also found under waterlogged soil an increase in growth rate in many of the accessions. This was statistically significant for 2017. In 2018 this was only significant for some of the accessions. In our trial, perennial ryegrass was very productive under flooding treatment and recovered very well to produce even higher biomass yield after flooding in many cases. This very good recovery and resilience of perennial ryegrass has also been shown by Oram *et al.* (2021) when it was waterlogged for three weeks and then allowed to recover for five weeks until harvest. Meehan *et al.* (2017) reported the same performance of perennial ryegrass under control, dry, and flooding conditions. In their analysis the significant differences from year to year were found which are comparable to the experiment reported here.

However, a significant reduction in biomass productivity under water excess conditions might be expected if the water stress was combined with heavy treading by livestock as observed by Nie *et al.* (2001). Similarly, mechanical damage by tractor tracks could reduce biomass yield under wet conditions when damaging soil structure up to 14.5% (Hargreaves *et al.* 2019).

There was no statistically significant difference in the first cut herbage DM production between treatments in 2018. On the other hand, herbage DM production was 0.589 t ha^{-1} higher on the waterlogged treatment compared with the non-waterlogged control in 2017. This difference can be explained, at least in part, by nitrogen applied with the irrigation water (1 620 mm in 2016) during the winter, which contained 10 kg ha^{-1} of N and 1 kg ha^{-1} of P. A typical herbage DM production response to additional N applied to grassland is approximately 33 kg ha^{-1} per kg of additional N (Humphreys *et al.* 2003, Murphy *et al.* 2013). Hence, an additional 10 kg ha^{-1} of N in irrigation water could potentially increase annual herbage DM production by approximately 0.33 t(DM) ha^{-1}, which would partly account for some of the difference between treatments in 2017. It is possible that the additional P in irrigation water could also have contributed to additional herbage DM production. However, taking into account the high soil test P status of the site during the experiment it is unlikely that the contribution of additional P in the irrigation water would have been substantially additive to annual herbage DM production over the contribution of the additional N. Obviously these nutrients would have no impact of herbage DM production during 2018; being taken up by the herbage DM during 2017.

The elemental composition of ryegrass agrees with previous studies for macro and micronutrients, albeit huge variation has been reported. For example, Crush *et al.* (1989) reported values of 0.27 - 0.28% for P, 3.25 - 3.49% for K, 0.44 - 0.51% for Ca and 0.21 - 0.22% for Mg and Crush *et al.* (2018) reported values of 66.4 - 127.2 mg kg^{-1} for Mn, 4.20 - 7.60 mg kg^{-1} for Cu and 0.53 - 0.97 mg kg^{-1} for Mo.

The macronutrients content in ryegrass showed differential variation with the water treatment: similar values for K, Ca, Mg, higher values for S, and lower values for P were observed in the flooded samples compared with control. The reasons for the increase of S and decrease in P content in waterlogged samples are not straightforward, since a decrease in soluble S and an increase in soluble phosphorus compounds when soil is flooded is expected (Ponnamperuma 1972). Nevertheless, previous studies have reported that phosphate availability to the plant is reduced by the waterlogging treatment in grasses and rice (Humphries 1962).

For the micronutrients under study, the content in flooded samples was lower compared to control samples, except for Zn. This may indicate a potential decrease in quality for some micronutrients under flooded conditions. Mo content exhibited the highest decrease due to flood, with less than half of the content observed in control samples, probably related with a decrease in soil availability under anoxic conditions, due to an increase of reducing Mo forms (Mo IV and V) in organic compounds that are usually less soluble and less available (Stiefel 2002) as well as by nutrient interactions in plant uptake, with the increase in plant sulphur absorption possibly inducing antagonistic effects on plant Mo uptake, while reductions in plant phosphorus content weakens the synergistic uptake of Mo by plants with nitrogen addition (Li *et al.* 2023).

Directions for the future contain to extend official cultivar testing to include testing under challenging environmental conditions. Since mixed swards have showed more resilience towards excess water, selection and breeding for enhanced performance of perennial ryegrass in species mixtures would help to enhance ecosystem benefits much more. Substantial variation among accessions in response to flooding suggests that in the future, breeding may play an important role to improve the resilience of perennial ryegrass to flooding. Holohan *et al*. (2019) showed an improvement in ecological adaptation of grassland species by selection in just a few generations. Detailed ecophysiological studies are necessary to determine the optimal water saturation of soil types for perennial ryegrass and the point in the physiology of the species from where excess water will be detrimental. It would be desirable to investigate variation in root growth under flooding and soil compaction conditions to select for accessions and within accessions for variation which can withstand these adverse conditions better. Decrease in root aerobic respiration rates is one of the earliest responses of plants when under flooded conditions and could be used to select for phenotypes to better withstand flooding conditions (Colmer 2003). Modern breeding tools like genome editing may be also a way forward to improve very directed tolerance of species towards adverse environmental conditions (Sustek-Sánchez *et al.* 2023).

However, the choice of species to suits is ecological niche needs to be kept in mind planting grasslands for certain ecological and geographical conditions.

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