Opposite metabolic responses of shoots and roots to drought

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Supplementary material

Material & Methods

Study site

Sampling was part of the EVENT II experiment[34], where precipitation patterns were experimentally modified in a semi-natural, extensively managed grassland in the Ecological-Botanical Garden of the University of Bayreuth, Germany (49°55'19" N, 11°34'55" E, 365 m a.s.l.). The climate is characterised as temperate and moderately continental with a mean annual air temperature of 8.2 °C and 724 mm of mean annual precipitation (1971-2000, data from the German Weather Service). The soil in the experiment is classified as a Gleysol. The homogeneous, loamy Ap horizon (42% sand, 43% silt, and 15% clay) has a depth of 30 cm, followed by a clayey Bg horizon. The water table drops to -1.5 to -2 m in summer and can reach -30 cm in winter and after longer periods of rain. The main rooting zone is within the upper 15 cm, and few roots penetrate the Bg horizon. The mean pH of the topsoil is 4.1 (1 M KCl). The volumetric soil moisture content for the permanent wilting point is near 15% and that for field capacity is near 40%. The experimental site is a semi-natural grassland that has neither been ploughed nor fertilised for at least 20 years prior to the installation of the EVENT II experiment in 2008. The meadow was mown twice a year for hay production prior to the start of the experiment. The semi-natural grassland community is dominated by tall grasses such as *Alopecurus pratensis* L. (meadow foxtail) and *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl (tall oat-grass) and harbours on average 16 species per m^2. All species are C3 plants.

Experimental design

The field experiment had a two-factorial design manipulating (1) variability of intra-annual precipitation (low, medium, and high, the latter including an extreme drought) and (2) warming (ambient, winter warming, and summer warming). The design consisted of 45 plots, each 1.5 × 1.5 m in size, with five replications of all factorial combinations. The warmed and unwarmed plots were blocked and randomly assigned within each manipulation of the precipitation.

The annual precipitation was kept constant since 2009, while the temporal pattern of precipitation in time was altered during the growing season (April-September). Three precipitation treatments, irrigated control, ambient control, and drought were established. The plots in the **irrigated control** treatment received at least the long-term (1971-2000) average weekly precipitation per week. The plots were exposed to ambient rainfall, but if the weekly rainfall was less than the long-term average, the deficit was added by irrigation. If the weekly ambient rainfall exceeded the long-term average, the excess was not subtracted for the next irrigation. The amount of precipitation in this treatment served as the reference amount for all other treatments. The plots in the **ambient control** treatment received ambient levels of precipitation plus four irrigations (before and after drought, six weeks after drought, and in late September near the end of the growing season) to compensate for the differences with the irrigated control treatment at those four times. The drought treatment also received these additions of water, so all three treatments received the same annual amount of precipitation. Rain was excluded from the plots of the **drought** treatment to simulate the local 1000-year recurrence of drought calculated by Gumbel statistics based on the 1961-2000 time series recorded at a local weather station. Drought was defined by the number of consecutive days with <1 mm daily precipitation and was simulated by the exclusion of natural rainfall for 42 days using rain-out shelters. The shelters had steel frames (Hochtunnel, E & R Stolte GmbH, Germany) and were covered with transparent plastic sheets (0.2 mm polyethylene, SPR 5, Hermann Meyer KG, Germany) during the period of simulated drought that permitted nearly 90% penetration of photosynthetically active radiation, based on tests prior to set-up. An 80-cm gap was left between the ground and the plastic sheets to allow air exchange near the surface, which reduced microclimatic artefacts such as increased temperatures or reduced wind speed. The amount of excluded rain was applied together with the adjustment to the irrigated control treatment at the end of the period of artificial drought as one heavy episode of rain within two days.

The warming manipulations were performed either during the winter (October-March) or the summer (April-September) starting in October 2009. Temperatures were increased using infra-red overhead heating lamps equipped with reflector domes (IOT/90 250W Elstein, Northeim, Germany) at a height of 0.8 m, theoretically providing 60 W/plot. The lamps were raised to 1 m when tall grasses reached 80 cm. Unwarmed plots were equipped with dummy heaters. The air temperature at 5 cm above the ground was raised on average by 0.9 °C in winter and by 1.3 °C in summer. Note that the focus of the study was the effect of drought, not of warming.

Target species

Two C3 grasses were selected as target species for this study: *A. pratensis* L. and *Holcus lanatus* L. Both species were selected based on their high frequency in the experimental plots and their importance in semi-natural grasslands across central Europe. *A. pratensis* is the dominant species at the experimental site, producing about 18% of the annual aboveground biomass. It is a tall (up to 110 cm) and productive species of agricultural importance in moist and nutrient-rich meadows. *H. lanatus* is also frequent at the site, but less productive (3% share of the annual aboveground biomass). It occurs in semi-natural grasslands throughout Europe, Asia, and North Africa and is invasive in North America and Australia. It tolerates a wide range of conditions but prefers moist meadows.

Collection and preparation of the tissue samples

Samples were collected at the end of the drought manipulation before irrigation in July and again at the end of the growing season in September. Above- and belowground tissues were sampled from each of the 45 plots (3 precipitation treatments × 3 warming treatments × 5 replicates). We collected a total of 347 samples. In July we collected 44 samples of leaf blades and 43 samples of fine roots of *H. lanatus* and 45 samples of leaf blades and 44 samples of fine roots of *A. pratensis*. In September we collected 42 samples of leaf blades and 41 samples of fine roots *of H. lanatus* and 45 samples of leaf blades and 44 samples of fine roots of *A. pratensis*. The samples were roughly cleaned and immediately frozen in liquid nitrogen. The procedure for sample preparation is described in detail by Rivas-Ubach et al. (2013)[35]. Briefly, the frozen samples were lyophilised and stored in plastic cans at -80 ºC. Soil contamination was removed from the root samples. Finally, the samples were ground with a ball mill (Mikrodismembrator-U, B. Braun Biotech International, Melsungen, Germany) at 1700 rpm for 4 min, producing a fine powder that was stored at -80 ºC until the extraction of the metabolites.

Elemental analysis

For the analysis of C and N, 1.5 mg of each powdered sample was analysed, and their concentrations were determined by combustion coupled to gas chromatography using a CHNS-O Elemental Analyser (EuroVector, Milan, Italy).

P, K, Fe, Mn, Mg, Ca, and S were analysed by extraction by acid digestion in a microwave reaction system under high pressure and temperature. Briefly, 250 mg of leaf powder were placed in a Teflon tube with 5 mL of nitric acid and 2 mL of H_2O_2. A MARSX press microwave reaction system (CEM, Mattheus, USA) was used for these acid digestions. The digested material was transferred to 50-ml flasks and resuspended in Milli-Q water to a final volume of 50 mL. The element concentrations were determined by ICP-OES (Optic Emission Spectrometry with Inductively Coupled Plasma) (Perkin-Elmer Corporation, Norwalk, USA).

Extraction of metabolites

Two sets of 50-mL centrifuge tubes were labelled as set A for liquid chromatography-mass spectrometry (LC-MS) analysis and set B for nuclear magnetic resonance (NMR) analysis. Each tube of set A received 150 mg of a powdered sample then 6 mL of water/methanol (1/1), and the samples were vortexed for 15 s and then sonicated for 2 min at room temperature. All tubes were centrifuged at 1100 × g for 15 min. Next, 4 mL of each tube of set A were transferred to its corresponding tube of set B. This procedure was repeated twice for two extractions of the same sample. The resulting extracts were used for metabolomic analysis.

Preparation of extracts for NMR analysis

Eight millilitres of the extracts were resuspended in water to reduce the proportion of methanol (<15% methanol). The solutions were lyophilised, and 4 mL of water were added to each tube, which was vortexed and centrifuged at 23 000 × g for 3 min. The samples were frozen at -80 ºC and lyophilised again. Finally, 1 mL of KD_2PO_4-buffered D_2O solution containing 0.01% TSP (trimethylsilyl propionic acid sodium salt) (pH 6.0) was added to each dried fraction. TSP was used as the internal standard for the NMR experiments. The solutions were transferred to 2-mL centrifuge tubes with a micropipette and centrifuged at 23 000 × *g* for 3 min, and 0.6 mL of the supernatants were transferred to NMR sample tubes.

The procedure for the extraction of metabolites is described in detail by Rivas-Ubach et al.[35].

Preparation of extracts for LC-MS analysis

Two millilitres of the supernatants of each tube of set A were collected using crystal syringes, filtered through 0.22 µm microfilters, and transferred to a labelled set of LC vials. The vials were stored at -80 ºC until the LC-MS analysis.

LC-MS analysis

LC-MS chromatograms were obtained with a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific/Dionex RSLC, Dionex, Waltham, Massachusetts, USA) coupled to an LTQ

Orbitrap XL high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with an HESI II (heated electrospray ionisation) source. Chromatography was performed on a reversed-phase C18 Hypersil gold column (150 × 2.1 mm, 3-μ particle size; Thermo Scientific, Waltham, Massachusetts, USA) at 30 ºC. The mobile phases consisted of acetonitrile (A) and water (0.1% acetic acid) (B). Both mobile phases were filtered and degassed for 10 min in an ultrasonic bath prior to use. The elution gradient, at a flow rate of 0.3 mL per minute, began at 10% A (90% B) and was maintained for 5 min, then to 10% B (90% A) for the next 20 min. The initial proportions (10% A and 90% B) were gradually recovered over the next 5 min, and the column was then washed and stabilised for 5 min before the next sample was injected. The injection volume of the samples was 5 µL. HESI was used for MS detection. All samples were injected twice, once with the ESI operating in negative ionisation mode (-H) and once in positive ionisation mode (+H). The Orbitrap mass spectrometer was operated in FTMS (Fourier Transform Mass Spectrometry) full-scan mode with a mass range of 50-1000 m/z and high-mass resolution (60 000). The resolution and sensitivity of the spectrometer were monitored by injecting a standard of caffeine after every 10 samples, and the resolution was further monitored with lock masses (phthalates). Blank samples were also analysed during the sequence. The assignment of the metabolites was based on the standards, with the retention time and mass of the assigned metabolites in both positive and negative ionisation modes.

NMR analysis

See the main text for the description of the NMR analysis. 1H NMR-based fingerprints were obtained for all samples. One-dimensional (1D) ^{1}H NMR spectra were acquired with suppression of the residual water resonance. The water-resonance signal was presaturated using a power level of 55 dB during a relaxation delay of 2 s. Each spectrum acquired 32 k data points over a spectral width of 16 ppm as the sum of 128 transients and with an acquisition time of 1.7 s. The total acquisition time was ~8 min per sample with a relaxation delay of 2s. All ^{1}H NMR spectra were phased and baselines were corrected and referenced to the resonance of the internal standard (TSP) at δ 0.00 ppm using TOPSPIN 3.1. See Rivas-Ubach et al. (2013) [35]for more details of the sampling and NMR determination. The data were subsequently used for the statistical analysis. A variable-size bucketing was applied to all ^{1}H NMR spectra with AMIX software (Bruker Biospin, Rheinstetten, Germany), scaling the buckets relative to the internal standard (TSP). The output was a data set containing the integral values for each assigned ^{1}H NMR spectral peak in the described pattern. The buckets corresponding to the same molecular compound were summed.

The NMR spectrometer described for the fingerprinting was used for the acquisition of the 2D NMR on selected representative samples. The probe temperature was set to 298.0 K, and TopSpin 2.1 (Bruker BioSpin) acquired and processed the experiments. The data for 1D ^{1}H NMR, 2D ^{1}H-^{1}H correlation spectroscopy (COSY), ^{1}H-^{1}H total correlation spectroscopy (TOCSY), ^{1}H-^{13}C heteronuclear single-quantum correlation (HSQC), and ^{1}H-^{13}C heteronuclear multiple-bond correlation (HMBC) were acquired using standard Bruker pulse sequences and routine conditions[35].

Statistical analyses

To test for differences in plant elemental stoichiometries and metabolomes between seasons and drought treatments, we conducted a PERMANOVA analysis of the LC-MS and NMR metabolomic fingerprints from *H. lanatus* and *A. pratensis* using Euclidean distances, with season (September and July), climatic treatment (ambient control, irrigated control, and drought), and organ as fixed factors. The number of permutations was set at 999. The PERMANOVA as well as PLS (partial least squares) analysis, and CIM (clustered image maps) conducted with R (R Development Core Team 2008).

The plant stoichiometric and metabolomic fingerprints were also subjected to principal component analysis (PCA) to understand how the stoichiometries and metabolomes of *H. lanatus* and *A. pratensis* shifted with the factors studied (organ, season, and climatic treatment). Fingerprints from leaves and roots for July and September were additionally submitted to separate PCAs (Fig. 2). The PCAs were performed by the *pca* function of the *mixOmics* package of R (R Development Core Team 2008). The score coordinates of the variables were subjected to one-way ANOVAs to find statistical differences among groups (see Supporting Information in Rivas-Ubach et al. (2013)). A Kolmogorov-Smirnov (KS) test was performed on each variable to test for normality. All assigned and identified metabolites were normally distributed, and any unidentified metabolomic variable that was not normally distributed was removed from the data set. Statistica v8.0 (StatSoft) was used to perform ANOVAs, post-hoc tests, and KS tests.

Figure caption

Figure S1. Plot of cases and variables in the PCAs conducted with the elemental, stoichiometric, and metabolomic variables in plants sampled in September. (A) Plot of cases and variables of shoots. (B) Plot of cases and variables of roots. C/N/P/K ratios are shown in red. The various metabolomic families are represented by colours: blue, sugars; green, amino acids; dark-green, amino-acid derivatives; yellow, related compounds to amino acid and sugar metabolism ; cyan, nucleotides; violet, osmolytes; and brown, terpenes and phenols. Metabolites: acacetin (Acace), adenine (Adenine), alanine (Ala), arginine (Arg), ascorbic acid (Asco), asparagine (Asn), aspartic acid (Asp), caffeic acid (Caff), catechin (Cate), chinic acid (Cin.acid), chlorogenic acid (CGA), choline (Choline), citric acid (Cit), disaccharide (Disac), galangin (Galangin), gamma-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glycine (Gly), glycine betaine (GB), glycine-alanine (Gly-Ala), hexose (Hexose), histidine (His), indol acetic acid (IAA), isoleucine (Ile), jasmonic acid (JA), kaempferol (Kaemp), lactic acid (Lac), leucine (Leu), limonene (Limonene), lysine (Lys), malic acid (Mal) mannose (Man), ocimene (Ocimene), phenolic group (Phenol), phenylalanine (Phe), proline (Pro), pyruvate (Pyr), quercetin (Quer), quinic acid (QA), resveratrol (Resv), sabinene (Sabinene), serine (Ser), shikimic acid (SA), tartaric acid (Tar), threonine (Thr), thymine (Thymine), tryptophan (Try). The means of cases are indicated by: blue, irrigated control; green, ambient control; and orange, drought. *Holcus lanatus* is indicated as *Holcus* and *Alopecurus pratensis* as *Alopecurus*.

Figure S2. Loading of elemental stoichiometric and metabolomic variables in the PC axes separating drought treatments (Fig. S1). Variables are coloured and labelled as described in the caption for Fig. 1. Asterisks showed statistical significance ($P < 0.05$) in one-way ANOVAs. The drawing is a reproduction of the painting by C. A. M. Lindman.

Figure S3. Clustered image maps of the metabolites of shoots in the different treatments. Based on the data of the PLS analysis. The red and blue colours indicate positive and negative correlations respectively.

Figure S4. Clustered image maps of the metabolites of roots in the different treatments. Based on the data of the PLS analysis. The red and blue colours indicate positive and negative correlations respectively.

Table S1-S4. One-way ANOVAs of all elemental stoichiometric and metabolomic variables for *Holcus lanatus* and *Alopecurus pratensis* for shoots and roots. For the NMR variables, marked by inverted commas (‘), integral mean value (mM) respect initial TSP (0.01%). For the LC-MS variables, marked by asterisks (*), integral mean value of deconvoluted total intensities. The statistically significant differences between treatments detected by Tukey’s HSD post-hoc tests are indicated by letters ($P < 0.05$) and bold type colour. Elemental stoichiometric C, N, P, and K concentrations and ratios and Fe, Mn, Mg, Ca, and S concentrations are shown in red. The various metabolomic families are represented by colours: dark blue, sugars; green, amino acids; dark green, amino-acid derivatives; yellow, realted compounds to the metabolism of amino acids and sugars; cyan, nucleotides; and brown, terpenes and phenolics. Metabolites: acacetin (Acace), adenine (Adenine), alanine (Ala), arginine (Arg), ascorbic acid (Asco), asparagine (Asn), aspartic acid (Asp), caffeic acid (Caff), catechin (Cate), chinic acid (Cin.acid), chlorogenic acid (CGA), choline (Choline), citric acid (Cit), disaccharide (Disac), galangin (Galangin), gamma-aminobutyric acid (GABA), glutamic acid (Glu), glutamine (Gln), glycine (Gly), glycine betaine (GB), glycine-alanine (Gly-Ala), hexose (Hexose), histidine (Hys), indol acetic acid (Indol.acetic), isoleucine (Ile), jasmonic acid (JA), kaempferol (Kamp), lactic acid (Lac), leucine (Leu), limonene (Limonene), lysine (Lys), malic acid (Mal) mannose (Man), ocimene (Ocimene), phenolic group (Phenol), phenylalanine (Phe), proline (Pro), pyruvate (Pyr), quercetin (Quer), quinic acid (QA), resveratrol (Resv), sabinene (Sabinene), serine (Ser), shikimic acid (SA), tartaric acid (Tar), threonine (Thr), thymine (Thymine), tryptophan (Try). The means of cases are indicated by: blue, irrigated control; green, ambient control; and orange, drought. *Holcus lanatus* is indicated as *Holcus* and *Alopecurus pratensis* as *Alopecurus*. IC, irrigated control; AC, ambient control; D, drought; SE, standard error.

Figure S1

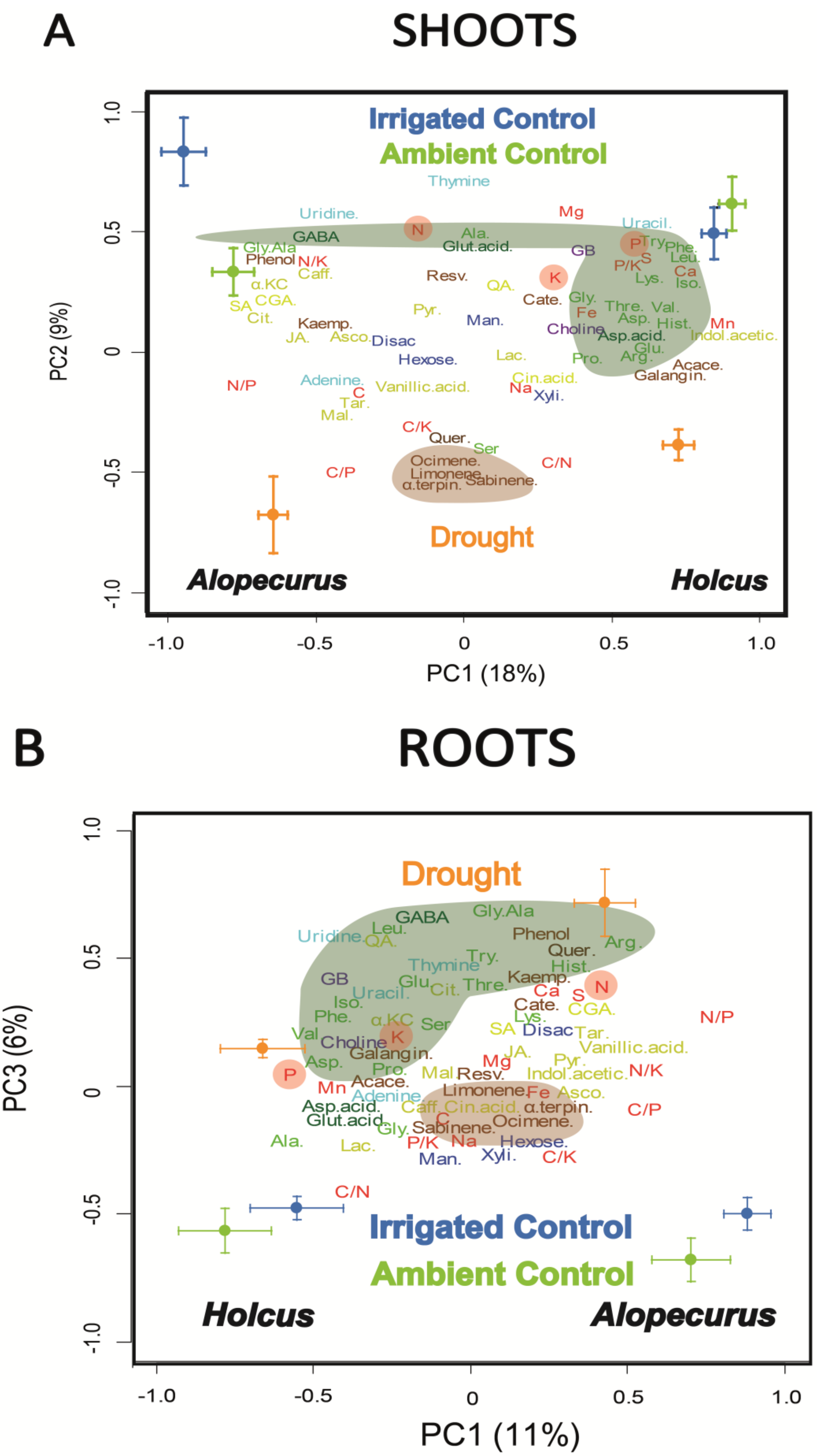
A
SHOOTS
Irrigated Control
Ambient Control
Drought
Alopecurus
Holcus
PC2 (9%)
PC1 (18%)
B
ROOTS
Drought
Irrigated Control
Ambient Control
Holcus
Alopecurus
PC3 (6%)
PC1 (11%)

Figure S2

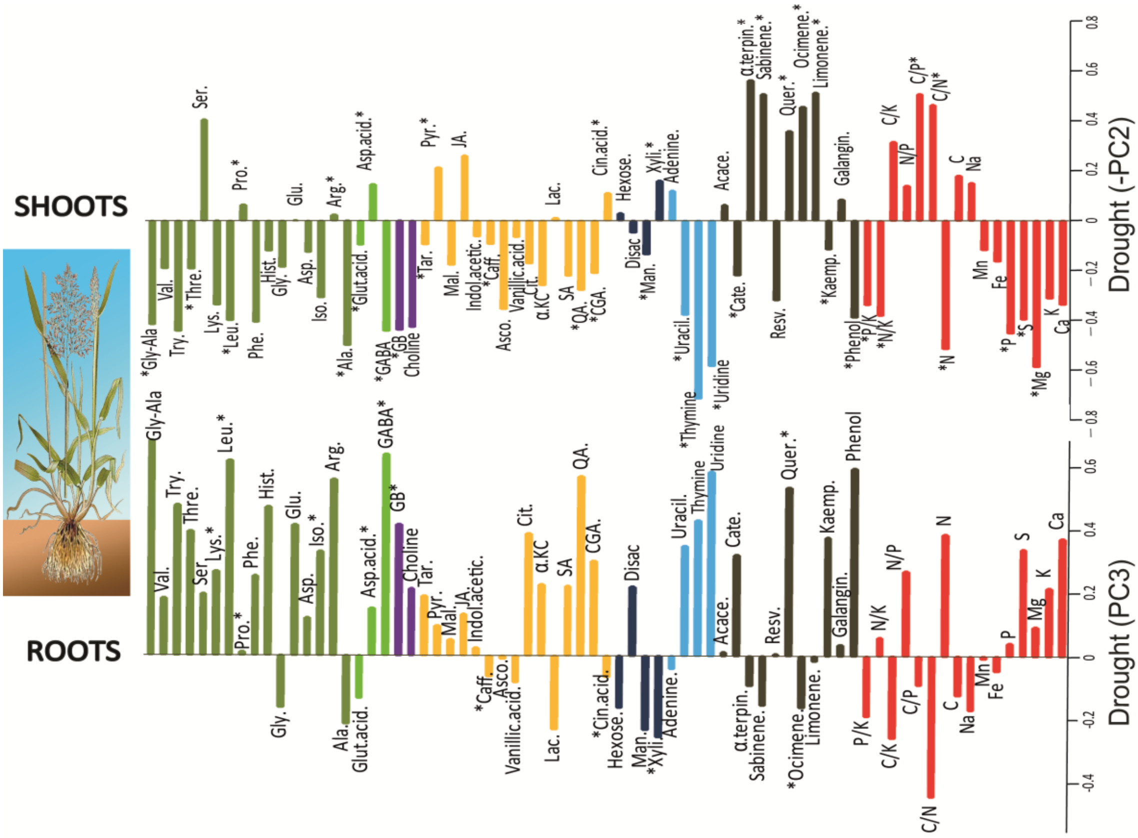

Figure S3

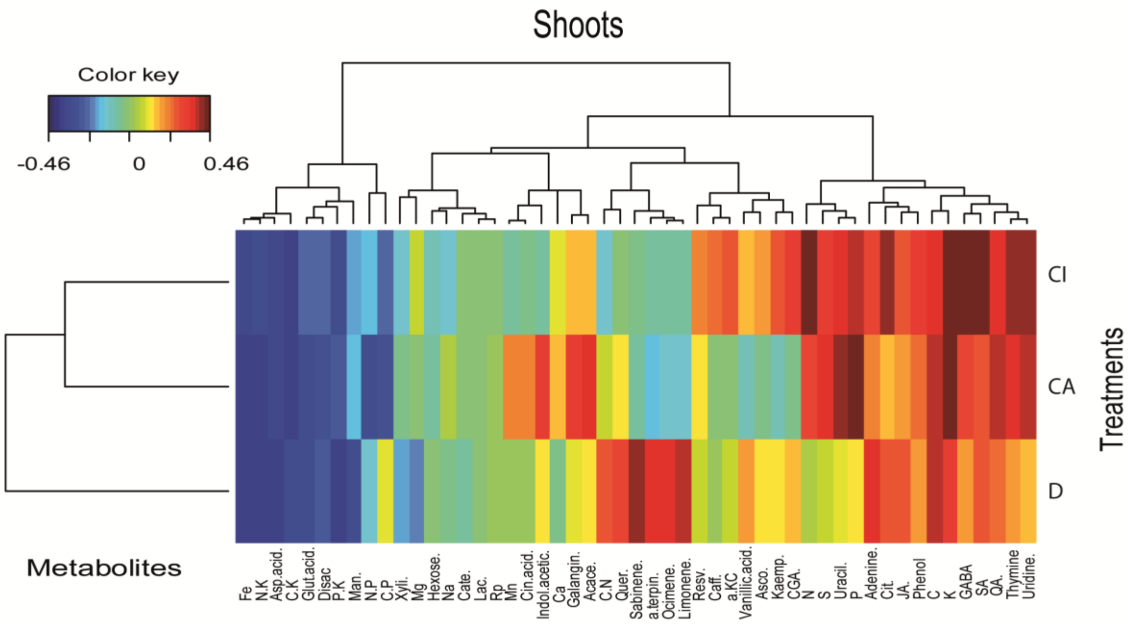
Shoots
Color key
-0.46
0
0.46
CI
CA
D
Treatments
Metabolites
Fe
N.K
Asp.acid.
C.K
Glut.acid.
Disac
P.K
Man.
N.P
C.P
Xyli.
Mg
Hexose.
Na
Cate.
Lac.
Rp
Mn
Cin.acid.
Indol.acetic.
Ca
Galangin.
Acace.
C.N
Quer.
Sabinene.
a.terpin.
Ocimene.
Limonene.
Resv.
Caff.
a.KC
Vanillic.acid.
Asco.
Kaemp.
CGA.
N
S
Uracil.
P
Adenine.
Cit.
JA.
Phenol
C
K
GABA
SA
QA.
Thymine
Uridine.

Figure S4

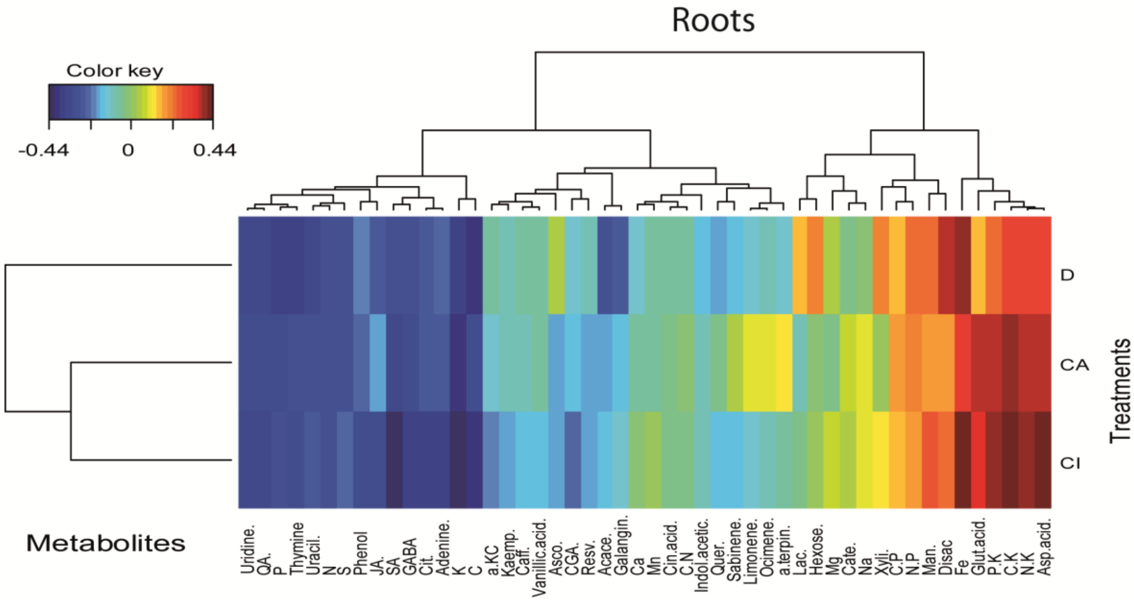
Roots
Color key
-0.44
0
0.44
D
CA
CI
Treatments
Metabolites
Uridine.
QA.
P.
Thymine
Uracil.
N
S
Phenol
JA.
SA
GABA
Cit.
Adenine.
K
C
a.KC
Kaemp.
Caff.
Vanillic.acid.
Asco.
CGA.
Resv.
Acace.
Galangin.
Ca
Mn
Cin.acid.
C.N
Indol.acetic.
Quer.
Sabinene.
Limonene.
Ocimene.
a.terpin.
Lac.
Hexose.
Mg
Cate.
Na
Xyli.
C.P
N.P
Man.
Disac
Fe
Glut.acid.
P.K
C.K
N.K
Asp.acid.

Table S1

	September																	
	Holcus lanatus																	
	Shoots									**Roots**								
Variable		IC	SE		AC	SE		D	SE		IC	SE		AC	SE		D	SE
Ala*		**5455431**	**624577**	**a**	**6639353**	**493427**	**b**	**3864623**	**397456**		**6750587**	**775560**		**10025088**	**1260622**		**7250622**	**801666**
Arg*		1448845	416475		1209846	300465		739206	131341		44010036	11786233		36190424	4732251		44841135	8914997
Iso*		2686151	465113		2423925	176064		2033295	219109		196396	56493		220851	89209		232366	60983
Asp*		2948222	790135		2541489	731817		2927460	660436		59858362	8163959		66039992	9467349		56416501	9734547
Glu*		6179981	1358115		7600689	1044119		5637589	700774		39687107	5465667		43904547	4542300		42488429	6671411
Gly*		98231	19386		85696	21092		48445	7486		500154	63513		616261	92449		552083	106649
Hist*		669726	173641		651973	139562		504570	92413		1958896	423251		2561680	666249		3453608	1046381
Phe*		54036325	10079646		44582844	4965201		39417286	3433630		3437841	706953		3868052	952576		4779895	1092501
Pro*		4607987	882696		5538016	799669		4166606	808808		7678912	1685327		7533019	1564328		7227301	956754
Leu*		59813541	8400371		58280471	3921053		45314490	3505937		7171776	1944186		9691050	3504836		5889631	1672844
Lys*		235067	62889		201504	36289		148370	17131		920860	354866		866942	235523		1159793	332619
Ser'		603620	83219		759698	62647		561877	48059		3092939	316180		3680457	502723		3178780	418300
Thre*		1617625	290899		1764917	176906		1294610	135770		6143189	559020		6349332	561841		7359756	807336
Try*		9793482	1650831		8219506	906220		7297803	729224		2019635	512423		2488897	493989		2093133	688109
Val*		40814944	5343724	a	46761649	2523763	b	30467341	2153153		26225073	3903888		26623730	3550549		30330018	2643822
Gly-Ala'	**a**	**0.332**	**0.026**	**a**	**0.326**	**0.017**	**b**	**0.23**	**0.011**		**0.174**	**0.017**		**0.162**	**0.007**		**0.199**	**0.028**
GABA'	**a**	**1.263**	**0.097**	**a**	**1.152**	**0.061**	**b**	**0.876**	**0.049**		**0.47**	**0.043**		**0.562**	**0.063**		**0.566**	**0.072**
Glu.acid*		23804619	2904783		23629189	1803661		18003391	1539546		66017523	4190089		78914864	8171632		79044423	9036087
Asp.acid*		1698053	334055		2055660	274088		1936930	223725		12536500	867457		12901067	709836		13130242	1110519
Choline'		7.156	0.571		6.841	0.639		6.031	0.514		3.877	0.257		3.599	0.306		3.624	0.234
GB		1.872	0.278		1.628	0.092		1.293	0.06		0.409	0.039		0.389	0.014		0.478	0.056
Lac*		4694190	538070		4312700	529887		3446182	337528		5073644	542814		4289147	497438		4790938	335722
α-KC'	**a**	**1422388**	**112990**	**a**	**1420664**	**70151**	**b**	**818217**	**107621**		**1544921**	**276213**		**1179973**	**119017**		**1248525**	**97969**
Cit*	**a**	**84036287**	**8167619**		**80713691**	**3521040**	**b**	**59695317**	**6409887**		**37589959**	**1689142**		**43488555**	**4529200**		**50414156**	**5581168**
Van.acd*		3396	570		2905	1058		4286	788		1045	187		820	103		993	139
Asco*		30743	4902		28712	5156		30052	5718		26571	3062		208602	81375		101516	48172

Caff*		40800	18633		18778	5149		50134	10155	a	2119	519	b	464	316	a	2156	383
Ind.acec*	b	6825121	1297755	a	14981674	1606183	b	5872145	1071198		1008198	151606		876357	188359		1219381	304645
JA*		**9616699**	**1539022**	**a**	**13058260**	**1579887**	**b**	**6777360**	**1424243**		**4369771**	**147898**		**2439761**	**935132**		**3383360**	**635873**
Mal*		**54809318**	**4955887**	**a**	**65126827**	**6770372**	**b**	**41122316**	**5954486**		**35170452**	**2653927**		**34331418**	**4760079**		**24032907**	**7481701**
Pyr*	**b**	**1448099**	**205592**	**a**	**2474932**	**355360**	**b**	**1406518**	**249547**		**1446319**	**416679**		**8108318**	**3037771**		**5686547**	**2069362**
Tar*		35745	8185		40308	7155		757405	466359		54449	4921	b	38036	26026	a	140042	42746
CGA*		16054258	4167074		4579973	1647834		10295418	2990894		975068	388414		15418873	8716145		11916170	11707045
QA*	**b**	**11222785**	**1317473**	**a**	**14291538**	**1384265**		**14822987**	**2661150**		**1281808**	**116707**		**1382891**	**85903**		**1560283**	**121679**
SA’	**a**	**0.32**	**0.02**	**a**	**0.31**	**0.01**	**b**	**0.25**	**0.02**		**0.1**	**0**		**0.11**	**0**		**0.12**	**0.01**
Cin acid*		7408	2553		3041	385		2448	330		1764	475		1126	91		1760	442
Xyli*		**2047084**	**374523**		**2433857**	**374431**		**2159874**	**315702**	**b**	**570148**	**118206**	**b**	**1030445**	**720628**	**a**	**3819043**	**980772**
Man*	**a**	**11639988**	**1778613**		**9152230**	**1223099**	**b**	**6711565**	**766968**		**13992699**	**682442**		**11696230**	**3586868**		**15430845**	**3042879**
Disac’		2659448	827430		2226574	996949		1568790	411188		14896661	1201890		15093457	1887800		15276314	1569367
Hexose*	**b**	**11401561**	**924384**	**a**	**21614456**	**1273569**	**c**	**7177151**	**1199880**		**14198373**	**718095**		**16704542**	**2664848**		**15803355**	**1276335**
Uridine*		**1561095**	**205568**	**a**	**1690784**	**186202**	**b**	**975113**	**105842**		**67568**	**4931**		**80563**	**2998**		**105250**	**21769**
Thymine’		13954526	559635		15316877	905232		13734843	657979		2413691	353504		3447244	358018		3097845	547322
Uracil*	**a**	**3531356**	**453544**	**a**	**3993568**	**322034**	**b**	**2273465**	**205522**		**12729**	**2417**		**19693**	**4493**		**10995**	**2413**
Adenine*		**2940441**	**318182**		**3027380**	**208455**		**2474247**	**106686**	**a**	**633446**	**46675**	**b**	**269024**	**77756**	**b**	**340244**	**62437**
Phenol’		**0.145**	**0.026**	**a**	**0.22**	**0.063**	**b**	**0.059**	**0.018**		**0.046**	**0.008**		**0.095**	**0.025**		**0.075**	**0.005**
Galangi*		**2757832**	**701519**		**4210309**	**707770**		**2264981**	**718975**	**b**	**449**	**72**		**848**	**201**	**a**	**1319**	**286**
Kaemp*		392000	67652		597713	219853		648873	148468		850	186		635	113		744	148
Limone*	**b**	**2217**	**345**	**a**	**4084**	**655**	**b**	**2263**	**326**	**b**	**1210**	**208**	**a**	**17976**	**6831**	**b**	**3427**	**740**
Ocimen*		**4690**	**1449**		**9824**	**2357**		**4675**	**871**	**b**	**2566**	**419**	**a**	**64858**	**27273**		**12027**	**6024**
Quer*		226265	107675		947025	300088		732522	339246		1335	466		614	286		2062	610
Resv*		6486	1036		5278	931		6572	987		3480	520		3684	709		3747	523
Sabinen*	**b**	**2398**	**403**	**a**	**5490**	**1038**		**4669**	**825**	**b**	**1307**	**212**	**a**	**19551**	**8366**		**3836**	**1267**
a-terpin*		**1692**	**275**		**2063**	**299**		**2057**	**349**	**b**	**864**	**143**	**a**	**18838**	**7054**		**6457**	**3981**
Cate*		5391965	1068238		7046830	2088802		7170013	1822377		608639	195703		7979989	2753367		9211987	3896121
Acace*		**173350479**	**31813040**		**254863936**	**29019444**		**183402611**	**25963933**	**b**	**14437**	**6960**		**43774**	**21435**	**a**	**87999**	**25361**
Ca		0.537	0.028		0.535	0.025		0.528	0.021		0.364	0.048		0.332	0.021		0.383	0.032
K		2.451	0.105		2.257	0.098		2.373	0.113		0.614	0.033		0.608	0.036		0.612	0.015
Mg		0.227	0.01		0.221	0.011		0.227	0.008		0.177	0.013		0.171	0.012		0.181	0.007
S		0.312	0.017		0.293	0.016		0.278	0.012		0.131	0.006		0.126	0.003		0.131	0.004
P		0.321	0.011		0.317	0.015		0.31	0.012		0.12	0.006		0.125	0.007		0.119	0.005

Fe		1.018	0.26		0.502	0.058		0.737	0.123		6.846	0.81		6.183	0.693		6.101	0.523
Mn		0.697	0.055		0.755	0.036		0.784	0.051		0.51	0.04		0.568	0.046		0.471	0.044
Na		0.258	0.1		0.225	0.074		0.128	0.023		0.224	0.036		0.203	0.027		0.148	0.011
C		43.3	0.3		44	0.2		43.6	0.3		37	0.9		38.7	0.6		38.1	0.9
N		1.75	0.07		1.67	0.07		1.63	0.07		1.04	0.05		1.02	0.04		1.04	0.04
C/N		39.8	4.2		38.2	2.5		53.5	6.2		42.2	1.3		38.9	1.4		41.9	2.2
C/P	**b**	**207**	**18**	**b**	**200**	**13**	**a**	**313**	**41**		**336**	**13**		**310**	**13**		**333**	**21**
N/P		5.44	0.27		5.3	0.24		5.95	0.34		7.98	0.2		8.03	0.34		8.02	0.41
C/K		21.4	1.8		21	1.5		24.3	1.8		61.8	4		56.2	3.4		53.3	4.3
N/K		0.565	0.029		0.552	0.019		0.487	0.031		1.475	0.098		1.462	0.104		1.297	0.096
P/K	**a**	**0.105**	**0.004**	**a**	**0.104**	**0.005**	**b**	**0.083**	**0.005**		**0.185**	**0.012**		**0.182**	**0.008**		**0.16**	**0.008**

Table S2

	September																	
	Alopecurus pratensis																	
	Shoots									**Roots**								
Variable		IC	SE		AC	SE		D	SE		IC	SE		AC	SE		D	SE
Ala*		**7572377**	**674946**	**a**	**9216009**	**764756**	**b**	**5347012**	**702880**	**a**	**10366122**	**1350641**	**a**	**10418354**	**851736**	**b**	**6391906**	**478989**
Arg*		372717	77114		343082	88773		463026	171892		101560409	15032972		140878319	20615519		126650420	20110959
Iso*		**1915150**	**318525**		**1919674**	**360070**		**1434047**	**165303**	**b**	**270934**	**84758**	**b**	**391254**	**76690**	**a**	**927321**	**193704**
Asp*		556647	202167		668653	190896		657108	210238		62789795	8137879		76707339	7675783		58553386	9099864
Glu*		3337800	490207		3140652	387983		2179904	349589		72398714	8502936		92176682	8892031		79290205	6812345
Gly*		92162	24124		72833	19774		71380	27864		284830	66721		338145	119172		227275	68253
Hist*		56802	11804		42742	13886		58836	14913		9709370	1841136		17972077	2991785		17595147	3446377
Phe*		**16453950**	**2370178**		**17891867**	**2469577**		**13858089**	**2428793**	**b**	**3404028**	**421423**	**b**	**4224345**	**553822**	**a**	**9442315**	**2378486**
Pro*		**6368062**	**1160727**		**6778407**	**994927**		**3622349**	**807927**	**b**	**21249561**	**2458040**	**b**	**26914543**	**5251231**	**a**	**22573458**	**3442706**
Leu*		**47128106**	**6256758**		**47062712**	**5173610**		**38545588**	**3910896**		**15613419**	**2941281**		**15038232**	**1812522**	**a**	**29221223**	**6260365**
Lys*		**153083**	**41808**		**107198**	**16731**		**76587**	**10909**	**b**	**1902829**	**482613**		**2994901**	**541022**		**5420794**	**1293365**
Ser'	**a**	**822639**	**78138**		**688766**	**106197**	**b**	**459560**	**65864**		**4300440**	**293695**		**4266948**	**189746**		**3977621**	**179655**

Thre*		1976269	192851	a	2160133	273405	b	1336627	149122		10205030	1151881		10156174	1126756		10227199	905509
Try*		3743871	467677		2995797	289025		2856758	229550	b	3439104	540632		5687921	827267	a	8169990	1875464
Val*	a	22849195	4554467		18607847	1195634	b	13619421	1192453		31726153	3616517		28755963	1843799		35127268	2618835
Gly-Ala'		0.432	0.024		0.384	0.018		0.408	0.019	b	0.208	0.017		0.247	0.022	a	0.273	0.017
GABA'		1.515	0.1		1.398	0.082		1.439	0.087	b	0.54	0.035		0.656	0.049	a	0.724	0.048
Glu.acid*	a	25251306	1348267		22813775	1635244	b	18100268	2170473		70340769	5282221		68440360	3546119		59075589	9190637
Asp.acid*		1378519	151958		1348719	238489		1175411	239541		14989931	789395	a	16130693	1246098	b	12505506	753090
Choline'	a	7.906	0.458	b	6.401	0.401		6.79	0.319		3.959	0.443		4.172	0.262		4.653	0.307
GB		1.081	0.087		1.018	0.081		0.975	0.053		0.435	0.03		0.524	0.042		0.604	0.065
Lac*	a	5599854	383671		4502833	454475	b	4238240	251878	b	3873338	470025	a	6829539	572155	a	5686787	336312
a-KC'		4064042	290066		3231990	312469		4160517	320661	b	1162989	109650		1599549	147641	a	2040850	186089
Cit*		131298694	12221827		115239885	8428440		121611533	8577289	b	34146430	2778997	a	49477761	4572587		38196145	3618848
Van.acd*		177267	107947		15475	4346		44356	17541		1528	223		1518	305		1227	105
Asco*	b	1229757	191624	b	3858731	1809204	a	11123293	2088670		42669	13208	a	56662	7316	b	8535	3499
Caff*		18458	2761		300967	131960		1201766	559270	b	5284	1718	a	101636	38332	b	406	217
Ind.acec*		904603	478853		65262	18158		425297	146656	b	8222	2518	a	10208130	3780032	b	8623	4173
JA*		11894814	1458547		14605350	1938150		11722683	1711688	b	1006920	158932	a	11025249	3015063	b	703507	346828
Mal*	a	128988859	11105557	b	78950501	8388005	b	77256459	3336267	b	24780986	5735540	a	55092859	10964353	b	26121979	2819533
Pyr*	b	2613649	457472		4593008	1654581	a	7572643	627420	a	1745666	309852	a	1728967	134390	b	672771	266632
Tar*	a	50714	10088		21460	4230	b	23297	7826		76856	26447		1130575	510165		344330	261134
CGA*		93726536	10860264		76707553	16524993		75181534	12338149	b	10823630	2578735		16076685	2183497	a	19829187	2906008
QA*		7511615	678430		6871627	518446		8834277	997018	b	1494173	82520		1873872	136994	a	2032097	152239
SA'		0.437	0.026		0.444	0.034		0.429	0.037	b	0.095	0.003		0.108	0.005	a	0.125	0.012
Cin acid*		1799	332		2930	207		3078	627		2039	619		2395	900		1851	331
Xyli*		1628301	134190	a	1755966	311053	b	936235	201822	b	977359	152045	a	4024122	316725	b	381660	198437
Man*	b	4281877	225901	a	7239618	240296	c	1921749	751123	a	12548016	2627738		10694587	922490	b	5608439	1480064
Disac'		5819064	1276810	a	7965761	1425752	b	2591909	544987	b	13271875	2099635	b	13493275	1270630	a	21947680	1134536
Hexose*	a	27348862	2268938		23780934	1596897	b	18734183	1352806		15546229	2144596		17976984	1066861		16213205	1639934
Uridine*		1246051	115708		1239193	151306		966210	58678	b	67624	4411		88197	5080	a	118728	16868
Thymine'		8063370	529474		7051695	590186		8875841	1327542		1514789	190418	b	1252484	185416	a	2092291	313635
Uracil*	a	2637213	199245		2106013	346466	b	1603573	222293		23461	5913		28857	4745		33704	5804
Adenine*	a	5667426	540743	b	4200037	278057	b	3962768	186394	b	254032	49170	a	1163841	178162	b	95611	40633
Phenol'		0.263	0.013		0.246	0.022		0.266	0.023		0.079	0.007		0.097	0.007		0.099	0.009
Galangi*		29821	9577	a	85003	33916	b	13795	4228	b	212	75		449	50	a	955	294

Kaemp*		**5573**	**1440**		**6443**	**2227**		**34746**	**15657**		**619**	**143**	**a**	**1135**	**333**	**b**	**119**	**72**
Limone*		2048	274		1206	145		17235	7764		1972	362		1722	305		1582	177
Ocimen*		**10425**	**1929**	**b**	**6952**	**998**	**a**	**31951**	**9891**		**3925**	**413**		**3218**	**515**		**4260**	**598**
Quer*	**b**	**22895**	**9725**	**a**	**7572818**	**1998202**	**b**	**2342062**	**1026639**	**b**	**394**	**37**	**a**	**1721534**	**650547**	**b**	**135**	**45**
Resv*		5228	925		4244	689		5109	568		3349	445		2238	246		2950	360
Sabinen*		4510	897		2248	334		17728	7811		1425	278		1558	199		1430	192
a-terpin*		1677	286		1278	103		8501	3113		929	144		1028	159		942	111
Cate*		**339801**	**67493**	**b**	**286171**	**33756**	**a**	**529182**	**72535**		**1789339**	**1687347**		**9829562**	**3548325**		**7379870**	**3401462**
Acace*		2121371	992474		6175414	2428878		2742925	1166878		4416	1658		2058	621		1845	567
Ca		0.306	0.023		0.292	0.017		0.332	0.009		0.374	0.024		0.323	0.014		0.372	0.021
K		2.137	0.048		2.209	0.046		2.151	0.086		0.603	0.034		0.604	0.018		0.711	0.042
Mg		0.155	0.005		0.137	0.009		0.161	0.009		0.169	0.008		0.162	0.007		0.179	0.01
S		**0.224**	**0.017**		**0.2**	**0.016**		**0.201**	**0.011**	**b**	**0.135**	**0.003**	**b**	**0.137**	**0.004**	**a**	**0.154**	**0.005**
P		**0.229**	**0.013**		**0.212**	**0.022**		**0.214**	**0.009**	**b**	**0.097**	**0.003**		**0.103**	**0.003**	**a**	**0.112**	**0.004**
Fe		0.387	0.169		0.228	0.031		0.445	0.146		6.139	0.477		5.801	0.39		6.241	0.523
Mn		0.323	0.04		0.367	0.035		0.375	0.033		0.461	0.048		0.511	0.043		0.615	0.072
Na		0.1	0		0.1	0		0.1	0		0.147	0.017		0.146	0.011		0.142	0.017
C	**a**	**45.4**	**0.3**	**b**	**43.7**	**0.2**	**b**	**44.3**	**0.3**		**36.3**	**0.6**		**35.8**	**0.6**		**36.9**	**0.7**
N		1.66	0.04		1.61	0.08		1.6	0.05		1.14	0.05		1.14	0.05		1.13	0.04
C/N	**b**	**34.3**	**1.6**		**37.4**	**1.1**	**a**	**40.9**	**1.5**		**32.8**	**1**		**33.6**	**1.4**		**33.6**	**1.1**
C/P		311	13		338	17		372	26		376	13		380	16		357	15
N/P		9.14	0.36		9.13	0.56		9.1	0.52		11.5	0.31		11.45	0.54		10.75	0.51
C/K		23.8	0.8		25.5	0.8		30.7	3.9		59	4.6		57.3	3.7		51.8	4.1
N/K		0.704	0.03		0.686	0.023		0.776	0.114		1.816	0.146		1.734	0.116		1.562	0.142
P/K		0.078	0.004		0.078	0.006		0.091	0.015		0.157	0.012		0.152	0.009		0.144	0.01

Table S3

	July																	
	Holcus lanatus																	
	Shoots									Roots								
Variable		IC	SE		AC	SE		D	SE		IC	SE		AC	SE		D	SE
Ala*	b	7318030	521211	a	8961091	383217	c	5645469	526691		13342817	1138047		12010155	1573799		14176059	1510350
Arg*	b	170716	16834		209135	26985	a	316220	66056		18067052	4389170		19726514	5462656		20324161	3191790
Iso*		2069390	214738	a	2665462	331565	b	1169995	268531	c	356573	79344	b	1182611	254488	a	179647	64644
Asp*	b	2302049	517102	a	6634759	1730245		3771146	763338		73748349	14726847		108776330	14465602		84195119	11014748
Glu*		3795371	435636		5285321	764162		6292320	1006188	b	61316221	6506272	b	72358358	8160513	a	98828574	7494726
Gly*		115788	21297		127146	21971		101995	23213	a	386585	90766	b	161768	35043	b	151808	21613
Hist*		187119	36133		267522	36598		245677	49673		1304499	583010		1527794	627434		950817	303684
Phe*	a	37473454	4761687	a	41018992	4708185	b	20267741	3441332		6214325	2189552	a	8209455	1634692	b	2179498	313530
Pro*		10880348	7151750		8428573	1654973		24745030	10053053	b	7608233	1213361	b	22183502	4652128	a	100886213	17464972
Leu*	a	54743428	4545048		67709526	6915826	b	36665733	6044448	b	17965148	2313670	a	36001178	5710761	b	12202000	2179337
Lys*		131211	19241		141613	27276		90210	13616	b	275484	38350	b	417734	107677	a	1035248	234998
Ser'		785529	80318		929142	105947		791490	70647		4293177	401154		5025670	727034		3961545	372318
Thre*	b	2068043	204216	a	3047090	310046	b	2030601	220855		7733206	918192		8419861	1058416		6605402	693997
Try*	b	5486162	639358	a	8524122	892114	b	4320799	644273		2774418	501953		3583317	453726		2758049	418088
Val*		24273700	1537281		32609468	3100677		27698003	3174191	b	29828716	3186436		33198703	3466738	a	40613251	2808314
Gly-Ala'	a	0.199	0.01	a	0.203	0.007	b	0.158	0.011		0.11	0.008		0.125	0.008		0.131	0.006
GABA'	a	0.759	0.062	a	0.747	0.057	b	0.492	0.037	c	0.353	0.017	b	0.427	0.025	a	0.504	0.019
Glu.acid*	a	21808054	1897866	a	23676374	1921045	b	14994892	1048022		64788771	4772637		59912939	4471082		52193343	4819893
Asp.acid*		1129440	200119		1936470	351597		1535936	220488		17938970	2082607		14221743	1076180		16040309	891575
Choline'		7.83	0.49		9.35	0.61		8.16	0.68		3.8	0.37	b	3.27	0.22	a	4.35	0.3
GB		0.907	0.054	a	1.084	0.059	b	0.734	0.052	b	0.318	0.022		0.399	0.03	a	0.504	0.046
Lac*		6637595	591856		7098668	527072		7779932	844853		7941343	938751		5889707	829818		9895397	1872333
a-KC'	a	1565783	117364	a	1791253	124417	b	1143422	113513		2966940	442705		3764830	870194		2551672	245893
Cit*	a	92001646	10019000		83542015	9145508	b	61973434	4065758		41996120	5031539		44731372	3831240		39969568	3436905
Van.acd*		1737	268		1864	314		1266	187		917	159		790	108		628	77
Asco*		56956	14063		58186	11415		89912	29224		174493	38326		273205	96089		169665	25339

Caff*		10790	1874		22119	6735		23486	8275	b	11598	3454	a	16689	4526	c	885	261
Ind.acec*	b	43032576	6367473	a	67643600	5696306		48976871	6486290		3439473	495944		3532470	602628		3034866	422087
JA*		10910675	1951833	a	16412422	2885960	b	8700608	1317939		4782235	611246		7446061	2640943		3569465	293017
Mal*		190709326	29670216		177125319	18340574		211930962	24454532		78163195	12979715		72114357	16195782		86428386	9549319
Pyr*		2359349	334812		4022579	559864		2613284	607448		1339989	232209		2134153	497300		3675881	1331409
Tar*		32663	5282		42507	8232		40110	9707		131923	35091		143984	36170		115135	33445
CGA*	a	4831126	1284514		3697966	805367	b	1358873	560226	b	515882	227356	a	17320256	7327665	b	1324616	352035
QA*	a	8165325	1059335	a	9197088	1186386	b	4379642	862134	c	1057451	65786	b	1387962	92803	a	1784458	116579
SA’	a	0.207	0.015	a	0.203	0.013	b	0.152	0.01		0.091	0.004		0.101	0.006		0.102	0.003
Cin acid*		3538	467		17218	7399		15715	11436	a	1714	249		2371	308	b	1321	212
Xyli*		1065071	354148		1113782	96582		1341665	203137		4221165	600699	b	2495916	457985	a	6332025	1279856
Man*		1835782	317562	a	3558336	908765	b	1331148	273889		19603927	2397185		16655623	1899713		17254123	1947221
Disac’		4027070	865106		4168078	814218		3381009	531249		7806540	1443939		8159888	1052520		12605883	1912680
Hexose*		29492615	3455187		35190387	1899620		32299843	3284412		39623704	4984250		43734635	2870173		56193469	6190221
Uridine*	a	429201	37790	a	450116	33454	b	185182	14931		59994	3959		75292	7183		67190	2878
Thymine’		5796447	790005		6326350	351554		4478895	560075		1214471	194493		938387	109901		1307293	188828
Uracil*	a	1030178	117069	a	1062511	118805	b	256070	80170		15078	3229		31224	11238		14235	2876
Adenine*		5508279	644403		5614837	711400		5225279	638711		1408810	218628		1892194	380503		1801907	163667
Phenol’	a	0.138	0.012	a	0.133	0.006	b	0.08	0.007		0.067	0.008		0.065	0.006		0.073	0.006
Galangi*		863787	205277		887013	178038		1292993	367792		5317	2546		5454	1573		2915	715
Kaemp*	b	54987	14544	a	130813	24136	b	16281	4717		852	230		926	216		617	119
Limone*		5539	1388		6771	1200		8864	3925		2590	454		3408	539		4019	669
Ocimen*		8268	1108	b	7158	1148	a	20533	6438	b	3966	615	b	4255	619	a	9075	1943
Quer*		402226	135019		2349354	1195583		499982	272556	a	92660	34970		62215	17676	b	679	175
Resv*		3735	438		4476	720		2849	297		2610	386		4036	579		4492	689
Sabinen*		6549	1129	b	5143	543	a	8893	1255		2656	424		4328	574		3327	468
a-terpin*		2344	446		2870	468		2949	584		1635	156		1837	267		2342	279
Cate*	b	449326	131928	a	1569190	449178	b	315960	84230		317752	60952		445608	85442		346511	41391
Acace*		116075751	28725029		107641048	17765723		162651406	28916030	b	1536487	772233	a	6327770	2252286	b	343892	177908
Ca		0.486	0.024		0.48	0.035		0.409	0.03		0.322	0.02		0.342	0.02		0.328	0.015
K	a	2.337	0.117	a	2.183	0.134	b	1.706	0.108		0.622	0.03		0.671	0.028		0.613	0.027
Mg	a	0.181	0.013		0.172	0.013	b	0.135	0.01		0.18	0.011		0.185	0.008		0.182	0.01
S	a	0.232	0.014	a	0.203	0.018	b	0.146	0.01		0.112	0.004		0.108	0.002		0.11	0.003
P	a	0.238	0.017	a	0.226	0.016	b	0.149	0.013		0.108	0.005		0.115	0.005		0.112	0.005

Fe		0.406	0.086		0.466	0.129		0.241	0.031		6.612	0.618		6.595	0.44		6.744	0.462
Mn		0.754	0.049		0.91	0.078		0.794	0.03		0.577	0.055		0.493	0.027		0.481	0.022
Na		**0.122**	**0.017**		**0.118**	**0.014**		**0.509**	**0.216**	**a**	**0.317**	**0.036**		**0.274**	**0.047**	**b**	**0.185**	**0.017**
C	**b**	**43.9**	**0.3**	**b**	**44.2**	**0.2**	**a**	**44.3**	**0.3**		**36.2**	**0.9**		**35.6**	**0.7**		**35.3**	**0.9**
N		1.336	0.106		1.196	0.066		0.826	0.075		0.877	0.036		0.901	0.025		0.915	0.023
C/N	**b**	**36.1**	**3.2**	**b**	**38.8**	**2.5**	**a**	**59**	**4.8**		**41.9**	**1.3**		**39.9**	**1.3**		**38.9**	**1.5**
C/P	**b**	**196**	**12**	**b**	**208**	**14**	**a**	**334**	**35**		**341**	**12**		**317**	**12**		**326**	**19**
N/P		5.636	0.277		5.384	0.204		5.698	0.338		8.186	0.259		7.975	0.255		8.339	0.259
C/K	**b**	**19.422**	**0.928**	**b**	**21.208**	**1.201**	**a**	**27.435**	**1.863**		**60.952**	**4.131**		**54.164**	**2.235**		**59.491**	**3.646**
N/K		0.566	0.028		0.554	0.021		0.485	0.03		1.481	0.119		1.362	0.047		1.519	0.056
P/K		0.101	0.004		0.105	0.006		0.089	0.007		0.18	0.012		0.172	0.007		0.184	0.008

Table S4

	July																	
	Alopecurus pratensis																	
	Shoots									Roots								
Variable		IC	SE		AC	SE		D	SE		IC	SE		AC	SE		D	SE
Ala*	**b**	**51635**	**12640**	**a**	**128123**	**24377**	**b**	**38207**	**6454**		**47506934**	**5533246**		**54907767**	**7857571**		**65384078**	**10212216**
Arg*		**362608**	**109951**	**a**	**456832**	**70043**	**b**	**143192**	**26607**		**205589**	**55456**		**385575**	**139868**		**228029**	**73860**
Iso*		1055640	745955		264010	170271		27201	5884		47907288	9169421		54297938	9481451		34678224	4102843
Asp*		**1481707**	**446173**	**a**	**2561565**	**470865**	**b**	**733572**	**152840**		**67710745**	**7248534**		**83464392**	**12732040**		**85491660**	**7177970**
Glu*		**85179**	**22675**		**79121**	**11239**		**46849**	**10065**	**a**	**118062**	**21762**		**180528**	**46320**	**b**	**62067**	**12450**
Gly*	**b**	**21879**	**6102**	**a**	**81506**	**16944**	**b**	**7090**	**916**		**2476781**	**604094**		**3098673**	**673351**		**2334828**	**845540**
Hist*	**a**	**14597074**	**3750617**		**11931423**	**1465539**	**b**	**5134975**	**1059096**		**1965845**	**441330**		**4641255**	**1651614**		**1875856**	**351320**
Phe*		**2601191**	**1398910**		**2591926**	**404298**		**2239842**	**601960**	**b**	**7374237**	**1259233**	**b**	**12691315**	**2217578**	**a**	**44541268**	**8600652**
Pro*		**25017073**	**3908106**	**a**	**31074315**	**4965113**	**b**	**14466561**	**3254798**	**a**	**16181970**	**2959552**		**29588202**	**7149487**	**b**	**13052201**	**2793730**
Leu*		33448	8335		63731	10650		31181	6981		660054	152481		1241837	387719		1129335	324085

Lys*		607529	98579	b	545099	64518	a	1786224	719912		4291613	548091		4550761	862643		3068763	494277
Ser’		1200606	188810		1395962	160828		1311212	308362		8353480	981111	b	10069047	1649752	b	5807935	771545
Thre*		3220744	659137		3324427	434396		1647376	372895	b	2493402	343951		4612905	750738	a	5141734	874697
Try*		11204410	1650204		16480336	3607708		7315032	1278450		24294521	2468845		26309239	2604252		23269818	2347528
Val*		0.321	0.023		0.304	0.018		0.241	0.025		0.127	0.012		0.171	0.029		0.178	0.019
Gly-Ala’		0.313	0.016		0.269	0.011		0.263	0.019		0.403	0.032		0.4	0.034		0.383	0.013
GABA’	a	31000875	4666754	b	19890800	1803059	b	15649731	1951109	a	40335500	3751264	a	40882961	4726560	b	28546667	2360199
Glu.acid*		264579	153261		23857	7065		262353	135251	a	2347	351	b	1037	165		1665	364
Asp.acid*		4656264	1417059		3414415	1261035		7647212	2524103	b	12911	4880	b	107111	19561	a	7005803	3312999
Choline’		0.71	0.046		0.841	0.09		0.568	0.073	b	0.263	0.031		0.349	0.064	a	0.423	0.04
GB		7797228	648499		7491770	779509		5933102	756801		6198626	361313	a	7360650	700348	b	5502606	443446
Lac*		8161540	1135337		6266173	1060876		5142950	720052		2439777	477057		1720260	244375		2021989	229832
a-KC’	a	207394614	15884979	b	152313932	13973238	b	151416246	11457041	b	31158028	3447209	a	54508357	6258352	a	48292221	4446748
Cit*	a	1.207	0.066		1.094	0.073	b	0.872	0.088		0.37	0.035		0.48	0.078		0.53	0.052
Van.acd*		914446	192157	a	1204315	202176	b	332623	81684		8563008	740407		11289242	1014315		9623591	827557
Asco*	a	9112347	3127518		5371990	851634	b	4584	943	b	0	0	a	1400	614	b	0	0
Caff*		91181	25570		8262193	5122108		130810	11709		1317	498		7731360	5679124		1143463	432188
Ind.acec*		27997334	3287789		26293370	2055700		32682448	2830901	b	1197198	452498	a	8327670	2616305	b	1538413	488467
JA*		277627203	33518594		388614365	38035978		418723224	80507256	b	9131236	3451283	a	70758121	14273455	b	16426295	6208556
Mal*	b	2736710	380794	a	6241151	926704	b	2594752	441261	a	32272625	9002369	b	984109	147761		14342592	6793949
Pyr*	b	60264	12980		91021	16431	a	130153	20891	b	115300	43579	b	859614	352831	a	4992432	1880652
Tar*		6.709	0.556		7.493	1.132		6.201	1.197		2.549	0.417		2.683	0.452		3.358	0.456
CGA*		6061276	536591		7313070	808693		6650127	1337627	b	1095909	123789		1393696	215968	a	1706388	181585
QA*		0.366	0.019		0.347	0.022		0.315	0.029		0.141	0.03		0.104	0.011		0.096	0.007
SA’		5557	2124		55625	29709		19732	6515		1262	242	b	521	135	a	1831	517
Cin acid*	a	65274595	12211132	b	8228695	3153148	b	29794348	4536558	b	178	67	a	2121	687	b	124	47
Uridine*	a	8696343	1091675	a	5890591	741746	b	2780520	801231	b	933091	100149	a	1671232	347553	b	909674	93448
Thymine’		176895	38617		197875	31653		195630	29658		12843	2449		17192	5407		7044	1062
Uracil*		7653031	752790		9680047	734166		10313898	1428797	b	305744	99410	a	969647	180175	a	1235014	228098
Adenine*		621017	98997		1208466	200357		824959	150797	a	5299801	1712125	b	1216981	326867	b	1557723	574427
Xyli*		2010061	331622	a	4066522	936892	b	535244	61512		9638055	1959177		14873802	3170440		16454037	2654546
Man*		5004646	1674833		7534803	859393		5651628	1024360		10706807	2252765		12048305	987812		16180379	1225328
Disac’		32525182	4500576		44631314	4871896		41543553	7710279		53670161	7299241		37118930	3210113		52514479	5846080
Hexose*		7109155	1050486		6634441	808963		5234967	917393	b	4246651	424803		3100053	589024	a	5165177	666248

Phenol’	a	304952387	26406629	b	118323205	14622706	b	80049018	11875291	a	24430696	4182682	b	11191593	1362627	b	14034454	1585868
Galangi*		2325	529		1945	441		2116	458	b	1172	148	b	1318	182	a	2585	409
Kaemp*	a	6638	918	a	6934	1245	b	51288	17824	b	2562	331	b	2536	320	a	4720	941
Limone*	b	17973	3997	b	10869	1569	a	191118	93359	b	4020	522	b	4006	512	a	6915	863
Ocimen*	b	460611	216894	b	365227	123099	a	11890732	1292867	b	109	41	a	568553	249327	b	72	17
Quer*		4348	817		4003	540		3698	657	b	2687	312	b	2474	405	a	4295	656
Resv*	b	7562	1396	b	4405	766	a	70119	19672		2517	536	b	1336	195	a	3176	673
Sabinen*	b	3626	492	b	2483	415	a	26183	7309		1687	237		2217	254		2473	409
a-terpin*		287685	59287		708829	164559		433064	58766	b	30551	18468	a	2058039	433031	b	124880	37289
Cate*	b	264916	134314	b	729108	298574	a	7411733	2906107		18129	9875		1214	280		1210	340
Acace*	a	644894	48506		546637	48679	b	432228	60203		51823	5195		68676	9268		51269	5039
Ca		1.886	0.043		1.793	0.042		1.904	0.065		0.599	0.028		0.606	0.03		0.633	0.024
K	a	0.165	0.012	b	0.118	0.008	b	0.106	0.011		0.19	0.011		0.184	0.012		0.186	0.012
Mg	a	0.129	0.01		0.122	0.006	b	0.102	0.005		0.122	0.003		0.122	0.004		0.131	0.003
S	a	0.151	0.005		0.136	0.007	b	0.122	0.011		0.091	0.003		0.098	0.004		0.096	0.003
P		0.192	0.03		0.211	0.028		0.14	0.014		7.015	0.471		6.607	0.372		6.357	0.528
Fe		0.292	0.021		0.339	0.028		0.302	0.02		0.429	0.029		0.402	0.028		0.456	0.032
Mn		0.1	0		0.116	0.016		0.1	0		0.239	0.023		0.216	0.022		0.176	0.017
Na		44.7	0.2		45	0.2		44.7	0.3		35	0.8		35.5	0.6		34.4	0.6
C	a	1.34	0.03	a	1.23	0.03	b	1.07	0.04		1.05	0.03		1.11	0.06		1.05	0.03
N	b	33.4	0.8	b	36.9	0.9	a	42.5	1.7		33.6	1		32.6	1.4		33	1.1
C/N	b	299	10		340	15	a	393	35		390	12		367	17		362	13
C/P		8.972	0.295		9.256	0.396		9.245	0.714		11.662	0.25		11.406	0.563		11.108	0.479
N/P		23.82	0.59		25.294	0.589		23.719	0.815		60.828	3.859		60.404	3.186		55.678	2.701
C/K	a	0.715	0.019	a	0.688	0.016	b	0.562	0.016		1.82	0.117		1.871	0.097		1.716	0.113
N/K		0.081	0.003		0.077	0.004		0.065	0.006		0.156	0.009		0.165	0.007		0.156	0.009
P/K		0.224	0.013		0.228	0.013		0.186	0.016		0.089	0.01		0.104	0.012		0.085	0.011