

A conservative resource use strategy in agricultural grasslands counteracts lower productivity and water use efficiency under drought conditions

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Soil characterization

Methods

Soil samples from a depth of 0-0.15 m were collected at three randomly selected locations per lysimeter, and these were then pooled to form a mix sample (approximately 500 g). These mix samples were air-dried, and sieved through a 2-mm sieve. Soil pH was measured potentiometrically in a solution of 0.01 M CaCl₂ (Thomas, 1996). Soil organic matter (SOM) was determined by weight loss after ignition in a muffle furnace at 550°C for 7 hours (Schlichting and Blume, 1995). The soil texture was measured with an automated particle size analyser (PARIO; Meter Group; Munich, Germany). Before the analysis, 30 g of air-dried soil from each lysimeter were treated with 30 % H₂O₂ to remove organic matter and were chemically (0.15 M Na₄P₂O₇) and mechanically (shaker) dispersed. The resulting suspension was analysed by PARIO, which calculates the particle size distribution by Stoke's law with a range spanning from 0.002 to 0.063 mm and provides a particle size distribution curve. Then, the suspension was flushed onto a cascaded sieve to obtain the particles between 0.063 and 2 mm. Particles smaller than 0.002 mm were calculated from the residual (i.e., unrecovered) mass. Particles were divided according to their size into sand ('large', 0.063 - 2 mm), silt ('medium', 0.002 - 0.063 mm) and clay ('small', < 0.002 mm) (after DIN 4022).

Results

Regarding soil parameters, SOM significantly decreased in all lysimeters by the end of the experiment from 6.03 ± 0.07 % to 4.88 ± 0.07 % (ANOVA, $F = 240.408$, $p < 0.001$), but no differences were seen between grassland types or irrigation levels. Soil pH (7.24 ± 0.03) did not change during the experiment. The analysis of soil particle size distribution revealed a sandy loam texture for all lysimeters. The particle size distribution in soil differed slightly between

wet and drought treatments by an increase on large particles (ANOVA, $F = 8.807$, $p = 0.041$) and a decrease of the share of fine particles (< 0.002 mm) in the top 15 cm for the wet treatment ($3.2 \% \pm 4.4 \%$ of fine particles) compared to the drought treatment ($9.6\% \pm 3.5\%$, ANOVA, $F = 6.224$, $p = 0.067$).

Results

Table S1 Result of all the ANCOVAs performed.

Variable	Effect	DFn	DFd	F	p	ges
SWC	Date	1	240	133.974	< 0.001	0.358
	Irrigation level	2	240	225.169	< 0.001	0.652
	Grassland type	2	240	38.870	< 0.001	0.245
	Date * Irrigation level	1	240	329.219	< 0.001	0.578
	Date * Grassland type	1	240	20.028	< 0.001	0.077
	Irrigation level * Grassland type	1	240	51.095	< 0.001	0.176
	Date * Irrigation level * Grassland type	1	240	27.731	< 0.001	0.104
ET	Date	1	736	11.336	0.001	0.015
	Irrigation level	2	736	243.480	< 0.001	0.398
	Grassland type	2	736	1.602	0.202	0.004
	Date * Irrigation level	1	736	609.696	< 0.001	0.453
	Date * Grassland type	1	736	0.357	0.550	0.0005
	Irrigation level * Grassland type	1	736	1.465	0.227	0.002
	Date * Irrigation level * Grassland type	1	736	0.591	0.442	0.001
Stomatal conductance	Date	5	2450	188.101	< 0.001	0.277
	Irrigation level	1	2450	1932.847	< 0.001	0.441
	Grassland type	1	2450	18.921	< 0.001	0.008
	Functional group	1	2450	457.019	< 0.001	0.157
	Date * Irrigation level	4	2450	250.274	< 0.001	0.29
	Date * Grassland type	5	2450	42.533	< 0.001	0.08
	Irrigation level * Grassland type	1	2450	28.656	< 0.001	0.012
	Date * Functional group	5	2450	23.017	< 0.001	0.045
	Irrigation level * Functional group	1	2450	143.159	< 0.001	0.055
	Grassland type * Functional group	1	2450	6.375	0.120	0.003
	Date * Irrigation level * Grassland type	3	2450	4.469	0.004	0.005
	Date * Irrigation level * Functional group	4	2450	20.68	< 0.001	0.033
	Date * Grassland type * Functional group	5	2450	6.68	< 0.001	0.013
	Irrigation level * Grassland type * Functional group	1	2450	8.053	0.005	0.003
	Date * Irrigation level * Grassland type * Functional group	3	2450	0.243	0.867	0.001
Stomatal conductance legumes	Date	5	1685	167.410	< 0.001	0.332
	Irrigation level	1	1685	1520.418	< 0.001	0.474
	Grassland type	1	1685	24.321	< 0.001	0.014
	Date * Irrigation level	4	1685	202.864	< 0.001	40.325
	Date * Grassland type	5	1685	37.777	< 0.001	0.101
	Irrigation level * Grassland type	1	1685	29.018	< 0.001	0.017
	Date * Irrigation level * Grassland type	3	1685	3.149	0.024	0.006
Stomatal conductance grasses	Date	5	765	26.082	< 0.001	0.146
	Irrigation level	1	765	426.182	< 0.001	0.358
	Grassland type	1	765	0.132	0.717	0.0002
	Date * Irrigation level	4	765	41.720	< 0.001	0.179

	Date * Grassland type	5	765	5.886	< 0.001	0.037
	Irrigation level * Grassland type	1	765	1.423	0.233	0.002
	Date * Irrigation level * Grassland type	3	765	1.698	0.166	0.007
Aboveground necromass	Date	1	104	168.444	< 0.001	0.618
	irrigation level	4	104	49.135	< 0.001	0.654
	grassland type	4	104	0.159	0.958	0.006
	Functional group	4	104	0.325	0.861	0.012
	Date * irrigation level	1	104	170.025	< 0.001	0.620
	Date * grassland type	1	104	0.061	0.806	0.001
	Irrigation level * grassland type	2	104	0.082	0.921	0.002
	Date * Functional group	1	104	1.059	0.306	0.010
	Irrigation level * Functional group	2	104	0.282	0.755	0.005
	grassland type * Functional group	2	104	0.053	0.948	0.001
	Date * irrigation level * grassland type	1	104	0.001	0.977	0.000
	Date * irrigation level * Functional group	1	104	0.242	0.624	0.002
	Date * grassland type * Functional group	1	104	0.0002	0.987	0.000
	Irrigation level * grassland type * Functional group	1	104	0.004	0.949	0.000
	Date * irrigation level * grassland type * Functional group	1	104	0.034	0.854	0.000

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43 Table S2 Results of all the ANOVAs performed.

Variable	Effect	Df	Sum Sq	Mean Sq	F	p
Log-aboveground phytomass	Irrigation level	1	1.09	1.09	15.613	0.001
	Grassland type	1	0.06	0.06	0.885	0.361
	Functional group	1	43.13	43.13	618.53	< 0.001
	Irrigation level * Grassland type	1	0.18	0.18	2.538	0.131
	Irrigation level * Functional group	1	4.37	4.37	62.658	< 0.001
	Grassland type * Functional group	1	0.8	0.8	11.443	0.004
	Irrigation level * Grassland type * Functional group	1	0.03	0.03	0.432	0.520
	Residuals	16	1.12	0.07		
Log-belowground phytomass	Irrigation level	1	4.812	4.812	30.232	< 0.001
	Grassland type	1	0.093	0.093	0.582	0.457
	Functional group	1	12.474	12.474	78.371	< 0.001
	Irrigation level * Grassland type	1	0.398	0.398	2.502	0.133
	Irrigation level * Functional group	1	12.317	12.317	77.383	< 0.001
	Grassland type * Functional group	1	0.038	0.038	0.239	0.631
	Irrigation level * Grassland type * Functional group	1	0.007	0.007	0.045	0.834
	Residuals	16	2.547	0.159		
Root:shoot ratio	Irrigation level	1	0.212	0.212	0.296	0.052
	Grassland type	1	3.174	3.174	4.423	0.052
	Functional group	1	28.058	28.058	39.102	< 0.001
	Irrigation level * Grassland type	1	0.246	0.246	0.343	0.567
	Irrigation level * Functional group	1	3.481	3.481	4.851	0.043
	Grassland type * Functional group	1	3.692	3.692	5.145	0.038
	Irrigation level * Grassland type * Functional group	1	0.144	0.144	0.2	0.661
	Residuals	16	11.481	0.718		
AMF spores abundance	Irrigation level	1	22.2	22.18	37.76	< 0.001
	Grassland type	1	0	0.01	0.018	0.894
	Size and pigmentation classes	8	750.3	93.79	159.689	< 0.001
	Irrigation level * Grassland type	1	0	0.02	0.034	0.853
	Irrigation level * Size and pigmentation classes	8	12.4	1.55	2.64	0.008
	Grassland type * Size and pigmentation classes	8	2.8	0.35	0.588	0.787
	Irrigation level * Grassland type * Size and pigmentation classes	8	3.6	0.45	0.774	0.626
	Residuals	260	152.7	0.59		
AMF spores evenness	Irrigation level	1	0.1	0.1	8.3	0.006
	Grassland type	1	0.1	0.1	4.3	0.045
	Irrigation level * Grassland type	1	0.02	0.02	1.2	0.27
	Residuals	36	0.5	0.01		
WUE _{ap}	Irrigation level	1	0.352	0.352	3.894	0.084
	Grassland type	1	0.180	0.180	1.988	0.196
	Irrigation level * Grassland type	1	0.557	0.557	6.157	0.038
	Residuals	8	0.724	0.090		
WUE _{tp}	Irrigation level	1	9.929	9.929	15.994	0.004

	Grassland type	1	0.186	0.186	0.299	0.599
	Irrigation level * Grassland type	1	2.587	2.587	4.166	0.075
	Residuals	8	4.967	0.621		

Table S3. Varimax rotated matrix after factor analysis and the grouping factors. The identified factors were categorized depending on the variables included as follows: Factor 1 = high plant productivity, factor 2 = generally high abundance of AMF spores, factor 3 = high productivity of grasses, factor 4 = high stomatal conductance and factor 5 = high abundance of large AMF spores.

Variables	Principal Components						Factor
	1	2	3	4	5	6	
Phytomass_total_total	0.877	0.325	-0.166	0.279	0.046	0.125	1
Phytomass_total_roots	0.869	0.387	-0.147	0.252	0.066	0.035	
Legumes_total_phyto	0.864	0.316	-0.219	0.290	0.053	0.130	
Legumes_root_phyto	0.854	0.367	-0.218	0.278	0.074	0.038	
Phytomass_total_above	0.844	0.201	-0.189	0.311	0.008	0.272	
Legumes_above_phyto	0.839	0.210	-0.210	0.298	0.013	0.287	
AMF_medium_black	0.810	0.364	0.102	-0.089	0.369	-0.188	
AMF_small_black	0.780	0.085	-0.476	-0.128	0.357	0.009	
ET_acc	0.702	0.280	-0.237	0.410	0.182	0.400	
Legumes_rootshoot	0.673	0.554	-0.299	0.273	0.100	-0.071	
AMF_big_brown	0.671	0.655	0.041	0.130	-0.152	0.225	
Ratio_grass_legu_above	-0.652	-0.397	0.438	0.035	0.172	-0.422	
AMF_small_yellow	0.289	0.911	-0.083	0.041	0.165	0.105	2
AMF_spores	0.313	0.906	-0.124	0.174	0.115	0.045	
AMF_evenness	-0.274	-0.860	-0.140	0.088	-0.158	-0.267	
AMF_medium_yellow	0.297	0.848	-0.192	0.365	-0.029	-0.073	
Phytomass_total_rootshoot	0.596	0.669	0.042	0.121	0.129	-0.112	
Grasses_gs_lastday	0.408	0.643	-0.310	0.497	-0.014	0.240	
SWC_end	0.357	0.590	-0.261	0.246	-0.162	0.581	
Legumes_gs_lastday	0.372	0.533	-0.338	0.479	0.073	0.418	
Grasses_total_phyto	-0.257	-0.029	0.889	-0.313	-0.131	-0.139	3
Grasses_root_phyto	-0.238	0.044	0.866	-0.410	-0.119	-0.042	
AMF_small_brown	0.228	-0.012	0.815	0.255	0.421	0.105	
Ratio_grass_legu_root	-0.533	-0.187	0.769	-0.260	-0.020	-0.109	
Ratio_grass_legu_total	-0.585	-0.235	0.703	-0.235	0.047	-0.179	
Grasses_above_phyto	-0.245	-0.341	0.665	0.233	-0.137	-0.517	
Grasses_gs_day1	0.151	0.282	-0.139	0.888	0.075	0.097	4
Legumes_gs_day1	0.401	0.083	-0.079	0.838	0.245	0.015	
AMF_medium_brown	0.350	0.454	-0.017	0.787	-0.116	0.115	
Grasses_rootshoot	-0.046	0.391	0.459	-0.687	0.052	0.307	
AMF_big_black	0.031	0.401	0.135	0.045	0.870	-0.169	5
AMF_big_yellow	0.339	-0.161	-0.336	0.223	0.724	0.395	

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50 Table S4 Results of linear regression analysis showing the effects of the identified factors on water use
 51 efficiency calculated as the aboveground phytomass per accumulated evapotranspiration (WUE_{ap}) and
 52 as the total phytomass, i.e. aboveground and belowground, per accumulated evapotranspiration
 53 (WUE_{tp}).

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.
		B	Std. error	Beta		
WUE_{tp}	(Constant)	3.794	0.074		51.136	< 0.001
	Factor 1	1.119	0.077	0.883	14.446	< 0.001
	Factor 2	0.475	0.077	0.375	6.127	0.001
	Factor 3	-0.062	0.077	-0.049	-0.804	0.452
	Factor 4	0.031	0.077	0.025	0.402	0.701
	Factor 5	-0.295	0.077	-0.233	-3.807	0.009
WUE_{ap}	(Constant)	1.725	0.066		26.248	< 0.001
	Factor 1	0.291	0.069	0.718	4.243	0.005
	Factor 2	0.018	0.069	0.044	0.263	0.801
	Factor 3	-0.034	0.069	-0.085	-0.501	0.634
	Factor 4	-0.058	0.069	-0.142	-0.838	0.434
	Factor 5	-0.216	0.069	-0.533	-3.153	0.020

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