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 % ± 4.4 % of fine particles) compared to the drought treatment (9.6% ± 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

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	

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

References

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