

10.1071/CP19502\_AC

© CSIRO 2020

Supplementary Material: *Crop & Pasture Science*, 2020, 71, 592–609.

**Plastic film mulching improves seed germination, seedling development and potential for perenniality of *Vicia unijuga* under subalpine climate conditions**

*Wei Tang*<sup>A,B</sup>, *Carol C. Baskin*<sup>C,D</sup>, *Jerry M. Baskin*<sup>C</sup>, and *Zhibiao Nan*<sup>A,B,E</sup>

<sup>A</sup>State key Laboratory of Grassland Agro-ecosystems, Lanzhou University, Lanzhou 730020, P. R. China.

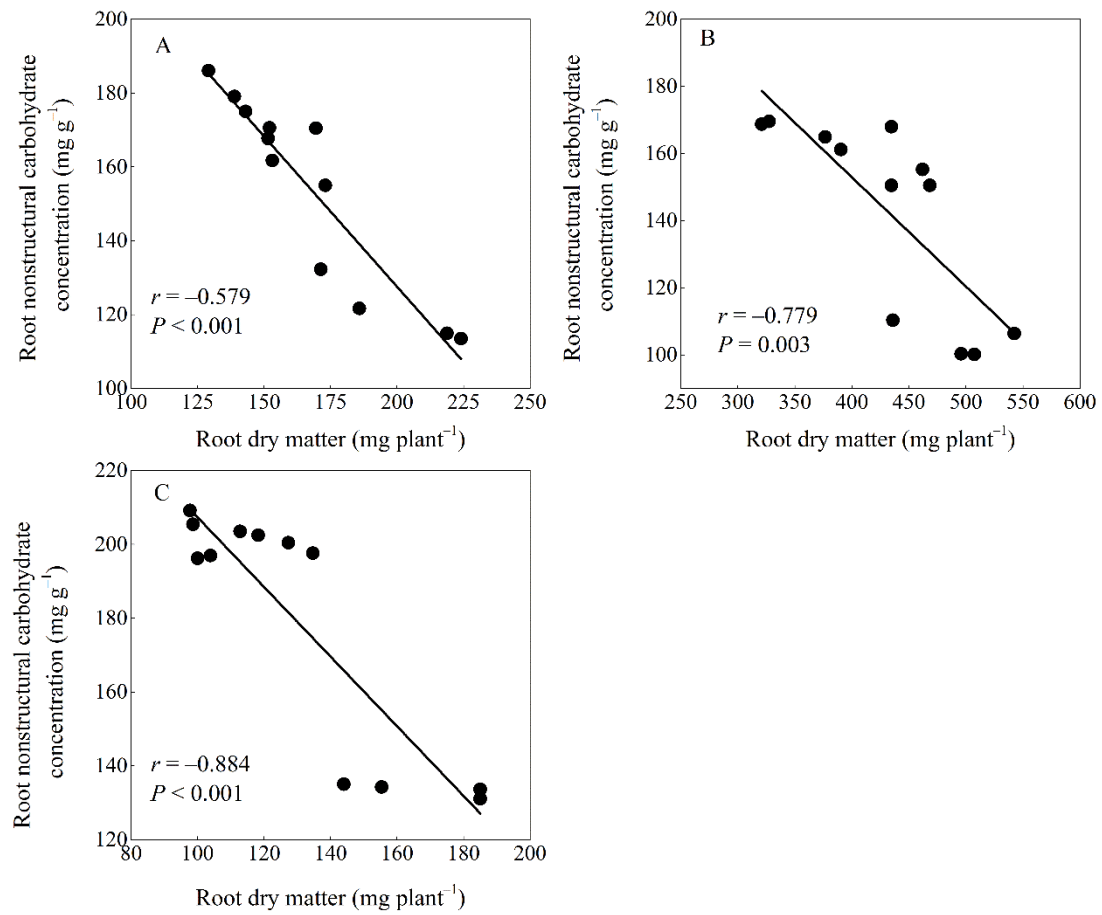
<sup>B</sup>College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu 730020, China.

<sup>C</sup>Department of Biology, University of Kentucky, Lexington, Kentucky 40506-0225, USA.

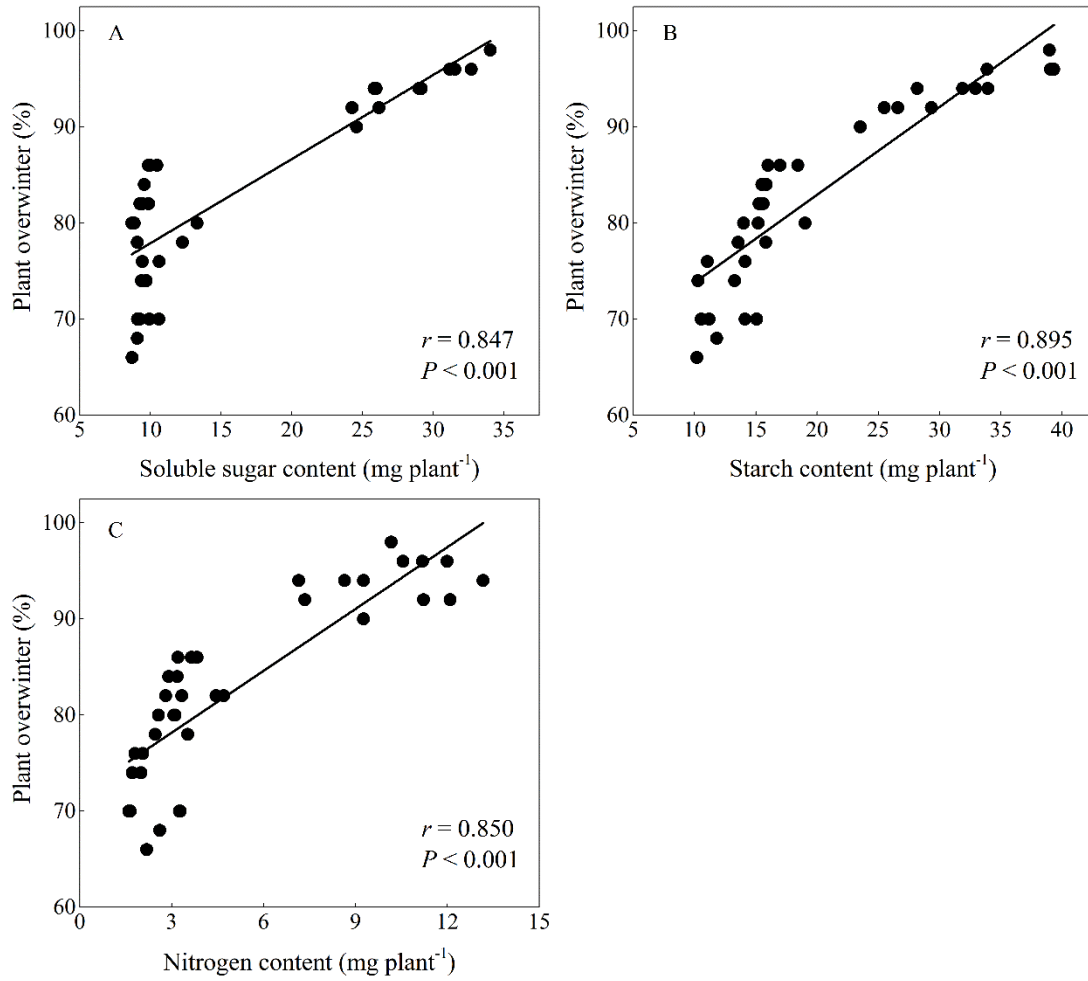
<sup>D</sup>Department of Plant and Soil Science, University of Kentucky, Lexington, Kentucky 40546-0312, USA.

<sup>E</sup>Corresponding author. Email: [zhibiao@lzu.edu.cn](mailto:zhibiao@lzu.edu.cn)

## Supplementary Figures



**Fig. S1.** Pearson's coefficients of correlation between root dry matter and root nonstructural carbohydrates (NSC) concentrations as influenced by the Control (A), and FM (B) and SM (C) treatment, respectively, during three years ( $n = 12$ ).



**Fig. S2.** Pearson's coefficients of correlation between soluble sugar content and plant overwinter percentage, starch content and plant overwinter percentage, nitrogen content and plant overwinter percentage as influenced by mulching treatment during three years ( $n = 36$ ).

## Supplementary Tables

**Table S1. Results of two-way ANOVA of effects of temperature (T), water potential (WP) and their interaction (T × WP) on final germination percentage (%) and germination rate (seeds<sup>-1</sup>) of *Vicia unijuga* seeds.**

Source	Final germination percentage			Germination rate		
	D.F. <sup>A</sup>	<i>F</i>	<i>P</i>	D.F. <sup>A</sup>	<i>F</i>	<i>P</i>
Temperature (T)	4	614.1	***	3	907.0	***
Water potential (WP)	5	395.8	***	4	475.4	***
Interaction (T× WP)	20	18.3	***	12	54.7	***

<sup>A</sup>D.F., degrees of freedom.

\*\*\*, *F*-value significant at  $P < 0.001$ .

Note: most germination did not reach to 20% at 5°C at all water potentials or at -1.0 MPa at 10 and 25°C. Thus, the results of germination rate from these conditions were not included in the two-way ANOVA.

**Table S2. Results of two-way ANOVA of effects of year (Y), mulching treatment (MT) and interaction of year and mulching treatment (Y × T) on shoot height (cm), root length (cm), shoot dry matter (mg plant<sup>-1</sup>), root dry matter (mg plant<sup>-1</sup>) and root:shoot ratio (mg mg<sup>-1</sup>) of *V. unijuga* plants.**

Source	D.F. <sup>A</sup>	Dependent variable				
		Shoot height	Root length	Shoot dry matter	Root dry matter	Root:shoot ratio
Year (Y)	2	8.3*	73.7***	4.4*	12.2**	200.0***
Mulching treatment (MT)	2	538.6***	1206.2***	544.5***	341.6***	164.4***
Interaction (Y×MT)	4	1.6 ns	0.4 ns	0.5 ns	1.1 ns	8.8**

<sup>A</sup>D.F., degrees of freedom.

Numbers are *F*-values significant at \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns = non-significant.

**Table S3. Results of two-way ANOVA of effects of year (Y), mulching treatment (MT) and interaction of year and mulching treatment (Y × MT) on *V. unijuga* root reserve concentration of soluble sugar (mg g<sup>-1</sup>), starch (mg g<sup>-1</sup>), nonstructural carbohydrate (mg g<sup>-1</sup>) and nitrogen (mg g<sup>-1</sup>).**

Source	D.F. <sup>A</sup>	Dependent variable			
		Soluble sugar	Starch	Nonstructural carbohydrate	Nitrogen
Year (Y)	2	160.1***	138.9***	187.5***	3.4*
Mulching treatment (MT)	2	65.1***	75.5***	84.5***	107.5***
Interaction (Y × MT)	4	6.7**	0.6 ns	3.1 ns	0.3 ns

<sup>A</sup>D.F., degrees of freedom.

Numbers are *F*-values significant at \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ ; ns = non-significant.

**Table S4. Results of two-way ANOVA of effects of year (Y), mulching treatment (MT) and interaction of year and mulching treatment (Y × MT) on *V. unijuga* root reserve content of soluble sugar (mg plant<sup>-1</sup>), starch (mg plant<sup>-1</sup>), nonstructural carbohydrates (mg plant<sup>-1</sup>) and nitrogen (mg plant<sup>-1</sup>).**

Source	D.F. <sup>A</sup>	Dependent variable			
		Soluble sugar	Starch	Nonstructural carbohydrate	Nitrogen
Year (Y)	2	6.3*	6.6**	7.7 **	4.4 ns
Mulching treatment (MT)	2	506.5***	151.0***	271.4***	235.9***
Interaction (Y × MT)	4	5.3 ns	2.6 ns	3.2 ns	0.8 ns

<sup>A</sup>D.F., degrees of freedom.

Numbers are *F*-values significant at \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ ; ns = non-significant.

**Table S5. Multiple linear regression analyses of *V. unijuga* root content of (A) nonstructural carbohydrate (NSC) and nitrogen (N); and (B) soluble sugar (SS), starch (St) and N with plant overwinter percentage as the response variable.**

A	Coefficients	S.E.	Lower 95 %	Upper 95 %	<i>t</i> -test	<i>P</i>	Variation explanation
Intercept	67.763	1.893	63.912	71.614	35.799	< 0.001	
Reserve NCS	0.324	0.110	0.100	0.549	2.941	<b>0.006</b>	<b>41.4%</b>
Reserve N	0.684	0.537	-0.409	1.778	1.273	0.212	36.7%
B	Coefficients	S.E.	Lower 95 %	Upper 95 %	<i>t</i> -test	<i>P</i>	Variation explanation
Intercept	64.338	2.096	60.069	68.608	30.696	< 0.001	
Reserve SS	-0.608	0.342	-1.305	0.089	-1.777	0.085	25.1%
Reserve St	1.197	0.322	0.540	1.853	3.714	<b>&lt; 0.001</b>	<b>32.2%</b>
Reserve N	0.812	0.490	-0.185	1.809	1.659	0.107	25.3%



**Table S6. Multiple regression analysis ( $Y = a_0 + a_1X_1 + a_2X_2$ ) of average soil temperature (ST, °C) at a depth of 10 cm from April to July ( $X_1$ ) and average soil water content (SWC, %) in the 0–20 cm depth from April to July ( $X_2$ ) during the three years of *Vicia unijuga* cultivation in various treatments on net leaf-level photosynthetic rate ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in July (A); of average soil temperature (ST, °C) at a depth of 10 cm from April to September ( $X_1$ ) and average soil water content (SWC, %) in the 0–20 cm depth from April to September ( $X_2$ ) during the three years of *V. unijuga* cultivation in various treatments on net leaf-level photosynthetic rate ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in September (B); The ST (°C) and SWC (%) were independent (explanatory) variables and the  $P_n$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) dependent (response) variables.**

The relative importance of the ST and SWC variables to these traits were examined through the separation of the contribution of each explanatory variable towards the  $R^2$  of the model.

Variables	(A) $P_n$ (July)			(B) $P_n$ (September)		
	Coefficient unstandardized	$R^2$	Relative importance	Coefficient unstandardized	$R^2$	Relative importance
Y	$a_0 = 3.13$	0.658***		$a_0 = -6.6$	0.622***	
$X_1$	$a_1 = 0.48$ ***		63.4%	$a_1 = 0.54$ ***		56.1%
$X_2$	$a_2 = 0.2$ ns		2.4%	$a_2 = 0.32$ *		6.1%

Results are taken from all mulching treatments across three growing seasons. \* and \*\*\* mean  $P < 0.05$  and 0.001, respectively; ns, no significant.