

Agriculture, Ecosystems and Environment

Soil properties after one year of interseeded cover cropping in topographically diverse agricultural landscape --Manuscript Draft--

Manuscript Number:	AGEE29252R3
Article Type:	Research Paper
Keywords:	Organic transition, Cover crop mixtures, Plant diversity, Interseeding in corn, Soil based ecosystem services, Topography, Maize.
Corresponding Author:	Linh Nguyen, PhD Michigan State University East Lansing, Michigan UNITED STATES
First Author:	Linh Nguyen, PhD
Order of Authors:	Linh Nguyen, PhD
	Kaleb Ortner
	Lisa Tiemann, PhD
	Karen Renner, PhD
	Alexandra Kravchenko, PhD
Manuscript Region of Origin:	UNITED STATES
Abstract:	Planting cover crops within or following a cash crop may improve soil-based ecosystem services due to increased plant diversity and a longer duration of live vegetation coverage. We examined the effect of three different cover cropping systems on soil properties after one year of a three-year organic transition rotation at four agricultural field sites with contrasting topographical positions, namely depressions, slopes, and summits. The four studied systems were (1) cereal rye (<i>Secale cereal L.</i>) planted after corn (<i>Zea mays L.</i>) harvest (Rye); (2) a mixture of cold susceptible cover crop species, namely, oat (<i>Avena sativa</i>), winter pea (<i>Pisum sativum</i>), and radish (<i>Raphanus sativus</i>), interseeded into corn (WK); (3) a mixture of cold tolerant cover crop species, namely, annual ryegrass (<i>Lolium multiflorum</i>), Dwarf Essex rapeseed (<i>Brassica napus</i>), and crimson clover (<i>Trifolium incarnatum</i>), interseeded into corn (WH); and (4) a no-cover control (NC). While soil moisture was affected by topography, interseeding cover crops into corn did not influence soil moisture levels at the 0-10 cm depth for the studied year. Soil NO₃ - content was markedly higher in the WK system compared to cereal rye and WH cover crop treatments. The difference was especially pronounced in depressions and summits. Soil N mineralization rates followed the pattern WH>WK>Rye>NC and the effects were most pronounced in slopes. Soil microbial biomass C was the highest in depressions followed by summits and slopes, and in depressions the WH had higher microbial biomass than the other systems. There were no effects of cover crops and topography on soil C mineralization one year after the organic transition was initiated. The WH system increased the fraction of 0.053-2 mm aggregates and decreased > 2 mm aggregates in depressions. The results suggest that the effect of cover cropping can become evident already one year after the organic transition begins. The WH mixture interseeded into the cash crop was an optimal cover crop choice for improving soil characteristics as well as decreasing soil N leaching risks during organic transition in undulating agricultural terrain. However, the magnitude of the benefit provided by WH was mediated by topography.
Suggested Reviewers:	Jennifer Blesh, PhD Associate Professor, University of Michigan jblesh@umich.edu Expert in the field
	Jason Kaye, PhD Distinguished professor, The Pennsylvania State University jpk12@psu.edu

	Expert in the field
	Jørgen Eivind Olesen, PhD Professor, Aarhus Universitet jeo@agro.au.dk Expert in the field
	Denise Finney, PhD Assistant Professor, Ursinus College dfinney@ursinus.edu Expert in the field
	Lammert Bastiaans, PhD Associate Professor, Wageningen University & Research lammert.bastiaans@wur.nl Expert in the field

Dear Editor,

We would like to thank you so much for your comments and suggestions for the manuscript. We made revisions for the highlights with detailed responses provided below.

On behalf of the co-authors,

Linh Nguyen

Editor's comments: I believe you have addressed nearly all the reviewer's comments and the manuscript is largely ready to be published. However, there is one prominent issue that still needs to be corrected. Using the treatment abbreviations in the highlights means that they are unintelligible to readers. Actually, abbreviations such as C and N are so widely used that they are acceptable in the highlights while treatment abbreviations are discouraged. Please find a way to state your highlights without using the treatment abbreviations. You can emphasize trends, i.e. since you have a diversity gradient consider reporting highlights with respect to that gradient or by the descriptor you used for the mixtures (i.e. cold tolerant vs cold susceptible). Once you have corrected this I will accept your manuscript for publication.

Thank you. We understand that abbreviations in the highlights should be avoided as they do not provide any information to readers. We made revisions for the highlight in which we use full name of the cover crop treatments as well as follow the requirement of characters (maximum 85 characters including space).

Highlights

- Cover crops did not affect soil moisture
- Cold tolerant mixtures decreased soil NO_3^- content
- Soil N mineralization rates were highest in response to cold tolerant mixtures
- Cold tolerant mixtures had higher microbial biomass C than the other systems.
- Cold tolerant mixtures changed soil aggregate size distribution in depressions.

Table 1 Baseline data for soil properties of different experimental fields at KBS at a depth of 0-20 cm. Bulk density measurements were not collected in field A-1.

Field	Depression						Slope						Summit					
	Soil texture			Bulk density (g/cm ³)	Total C (%)	Total N (%)	Soil texture			Bulk density (g/cm ³)	Total C (%)	Total N (%)	Soil texture			Bulk density (g/cm ³)	Total C (%)	Total N (%)
	Sand (%)	Silt (%)	Clay (%)				Sand (%)	Silt (%)	Clay (%)				Sand (%)	Silt (%)	Clay (%)			
82-1	65.4	26.5	8.1	1.62	0.86	0.03	74.1	16.2	9.7	1.52	0.58	0.02	63.4	27.9	8.6	1.78	0.71	0.03
MP	56.7	30	13.3	1.52	0.97	0.09	64.3	20.8	14.9	1.52	0.58	0.05	57.8	33.8	8.4	1.57	0.68	0.07
A-1	55.4	30.6	7.9	-	0.82	0.06	55.9	31.1	12.9	-	0.65	0.07	60.6	35.8	3.3	-	0.69	0.07
85	18.4	62.1	19.5	1.38	1.39	0.14	65.3	19.9	14.8	1.68	0.75	0.08	54.6	30.8	14.6	1.67	0.96	0.09

Table 2. Pearson correlation coefficient between soil properties and cover crop (CC), weed, and total aboveground plant biomass in fall and spring at each topographical position separately and in all topographies together. Bold and italic fonts mark correlation coefficients significantly different from zero at $P < 0.05$ and $P < 0.1$, respectively.

Variable	Depression			Slope			Summit			All topographies together		
				Fall								
	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass
Soil moisture	-0.255	-0.139	-0.107	-0.089	0.151	-0.013	0.185	0.042	0.087	0.036	0.013	0.064
Soil NH ₄ ⁺ content	0.240	0.423	0.138	-0.230	0.084	-0.093	0.360	-0.065	0.090	0.173	0.086	0.033
Soil NO ₃ ⁻ content	-0.048	0.160	0.088	-0.147	0.139	-0.007	-0.227	-0.160	-0.179	-0.034	0.031	0.034
Soil N mineralization rate	-0.064	0.014	0.067	0.175	-0.034	0.219	0.082	-0.324	-0.052	0.036	-0.141	0.072
Soil C mineralization rate	-0.110	-0.273	-0.222	0.316	0.180	0.225	0.093	0.145	0.087	0.043	0.035	-0.008
Soil microbial biomass C	0.238	-0.141	0.145	-0.056	-0.007	-0.040	0.010	0.065	-0.027	0.151	-0.014	0.110
Aggregate >2mm	-0.468	0.206	-0.315	0.270	-0.034	0.124	-0.046	-0.115	-0.134	-0.169	0.026	-0.155
Aggregate 0.25-2mm	0.516	-0.186	0.342	-0.271	0.037	-0.120	0.107	0.195	0.197	0.193	0.010	0.175
Aggregate 0.053-0.25 mm	0.328	-0.157	0.187	-0.271	-0.005	-0.150	-0.046	-0.067	-0.026	0.009	-0.096	0.004
Variable				Spring								
	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass
	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass	CC	Weed	Total plant biomass
Soil moisture	0.017	-0.197	-0.219	-0.153	-0.275	-0.191	-0.493	0.013	0.123	0.007	-0.042	-0.047
Soil NH ₄ ⁺ content	-0.175	0.433	0.294	-0.102	0.057	-0.059	0.141	0.362	0.432	-0.002	0.259	0.206
Soil NO ₃ ⁻ content	-0.339	-0.248	-0.404	-0.073	-0.280	-0.336	-0.531	0.061	-0.264	-0.286	-0.051	-0.280
Soil N mineralization rate	-0.006	0.064	0.089	0.307	-0.269	0.165	0.050	-0.249	-0.140	0.134	-0.122	0.016
Soil C mineralization rate	-0.017	-0.426	-0.456	0.118	-0.051	0.060	0.000	-0.508	-0.402	0.041	-0.381	-0.295
Soil microbial biomass C	0.082	-0.124	-0.042	-0.031	-0.184	-0.078	-0.086	0.237	0.092	-0.027	0.030	0.021
Aggregate >2mm	-0.249	0.257	0.006	0.178	0.097	0.207	-0.047	0.176	0.143	0.015	0.094	0.054
Aggregate 0.25-2mm	0.290	-0.257	0.031	-0.151	-0.098	-0.176	-0.024	-0.283	-0.239	-0.019	-0.146	-0.081
Aggregate 0.053-0.25 mm	0.100	-0.191	-0.061	-0.226	-0.084	-0.251	0.051	-0.142	-0.135	-0.046	-0.097	-0.115

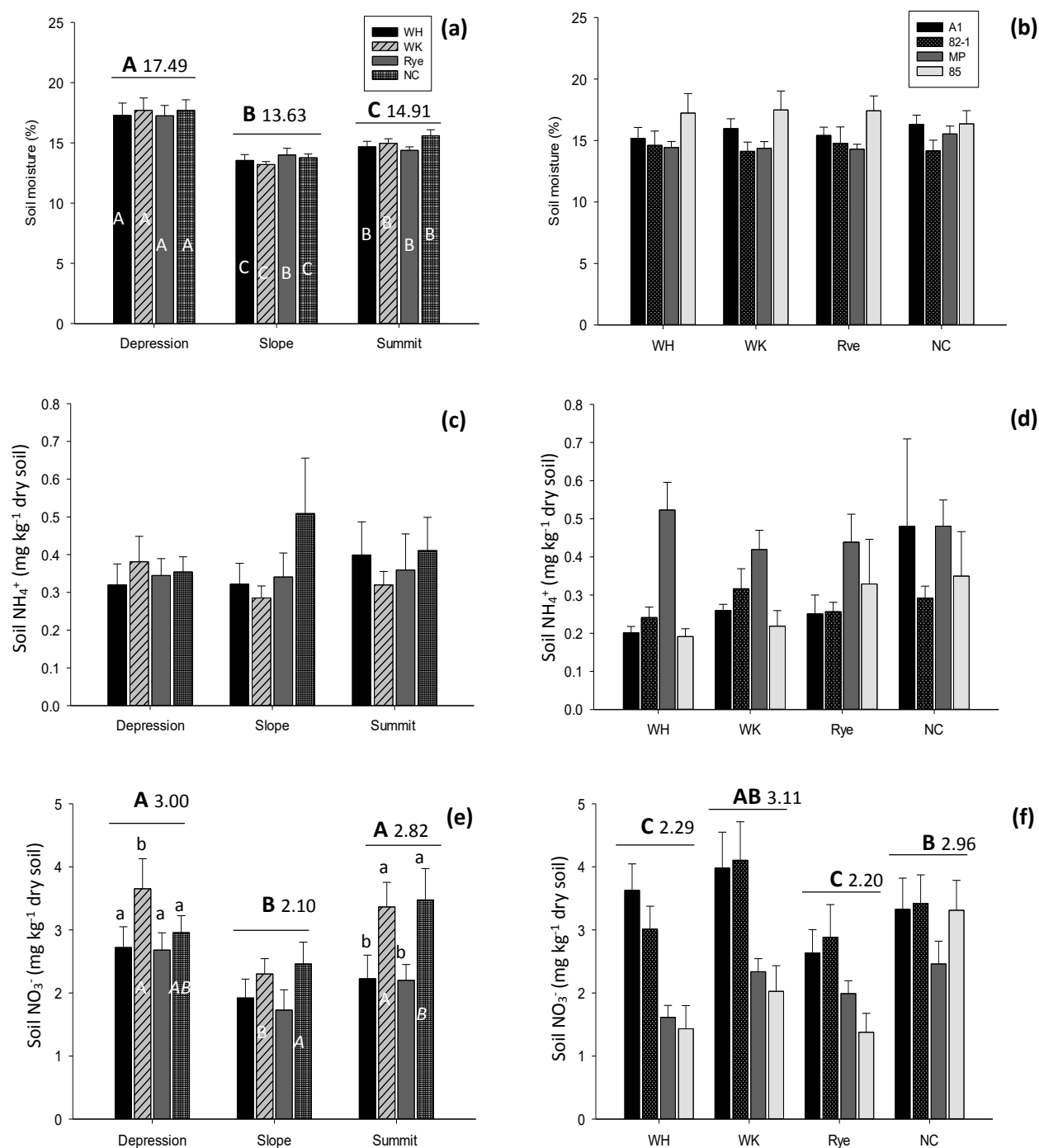


Fig.1. Soil moisture (a & b), soil mineral NH_4^+ (c & d) and NO_3^- (e & f) contents at 10 cm depth for the studied cover crop systems and topographical positions across all fields (left side graphs) and at the studied cover crop systems in individual fields across all topographical positions (right side graphs). Shown are means and standard errors. White regular and italic capital letters indicate statistically significant differences among topographical positions within each cover crop treatment at $P < 0.05$ and $P < 0.1$, respectively. Lowercase letters indicate statistically significant differences among cover crop systems within individual topographical positions ($P < 0.05$). Bold capital black letters mark significant differences

among marginal means of topographical positions (left side graphs) and of cover crop systems (right side graphs) ($P < 0.05$).

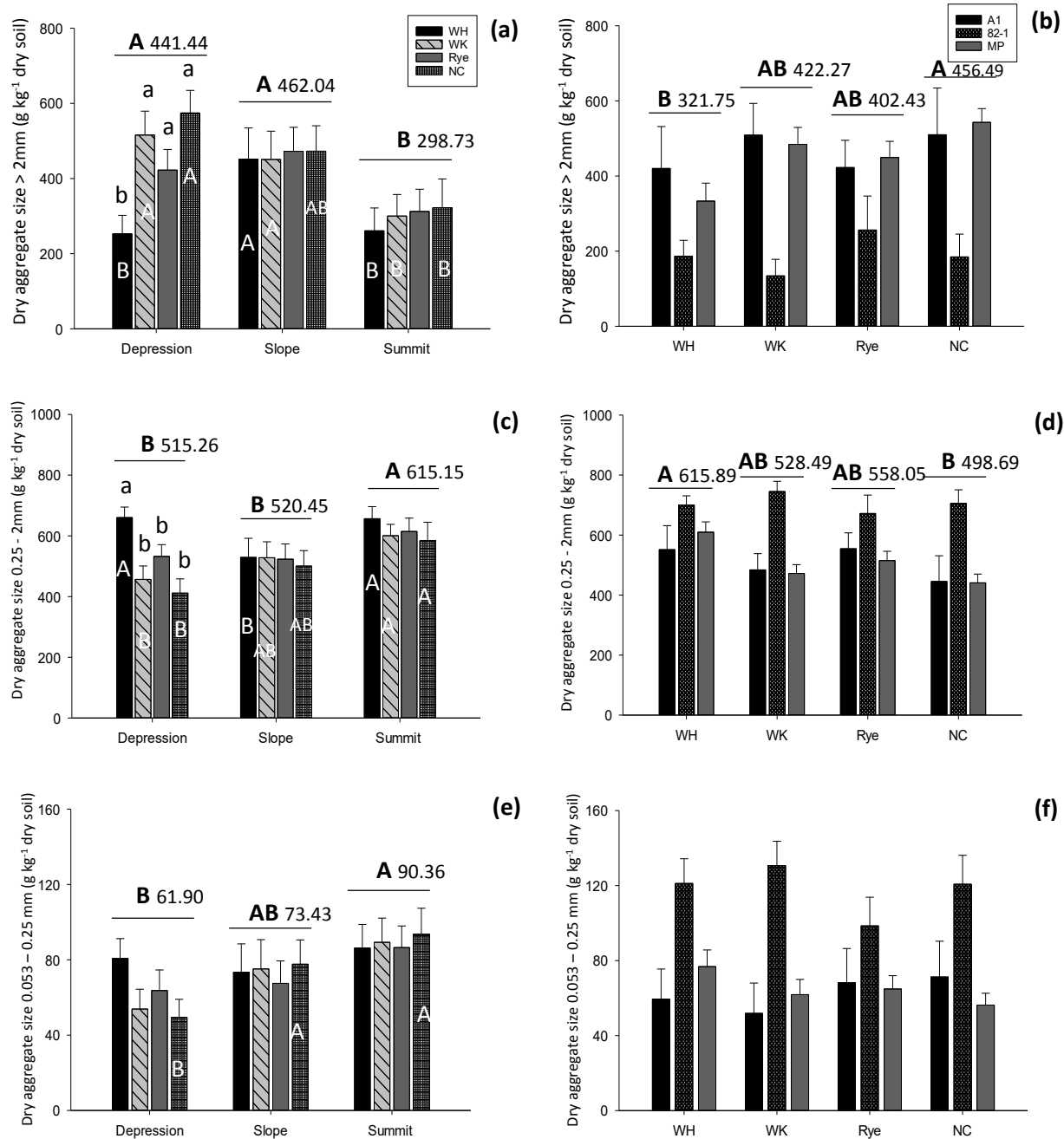


Fig.2. Mean weights of the three studied aggregate fractions at 10 cm depth for the studied cover crop systems and topographical positions across all fields (left side graphs) and at the studied cover crop systems in individual fields across all topographical positions (right side graphs). Shown are means and standard errors. White regular and italic capital letters indicate statistically significant differences for each cover crop treatment across three topographical positions at $P < 0.05$ and $P < 0.1$, respectively. Lowercase letters indicate statistically significant differences among cover crop systems within individual topographical

positions ($P < 0.05$). Bold capital black letters mark significant differences among marginal means of topographical positions (left side graphs) and of cover crop systems (right side graphs) ($P < 0.05$)

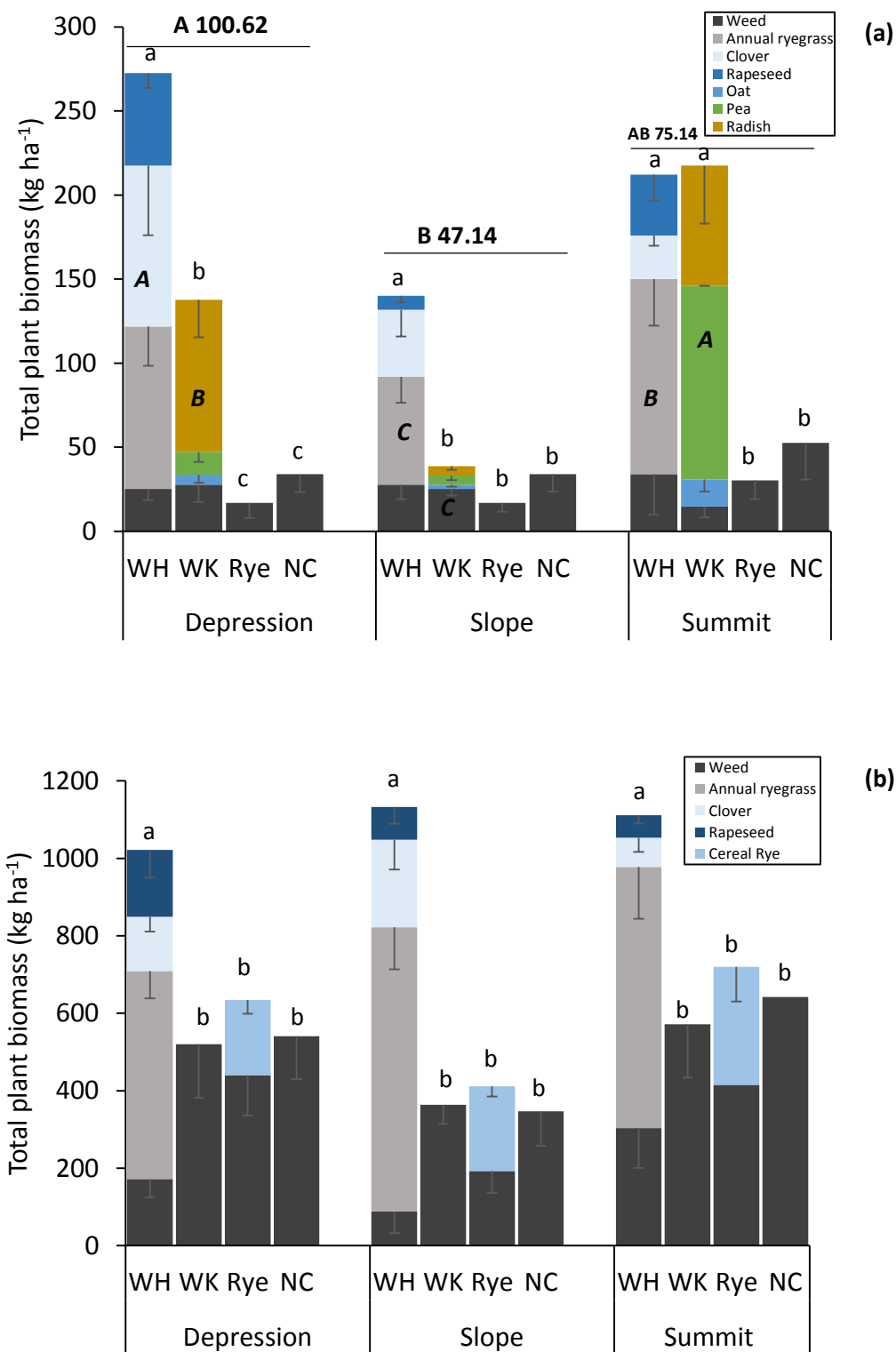


Fig. 3. Average total plant biomass (cover crop and weed) in fall (a) and spring (b) for all fields together. Lowercase letters indicate significant differences among cover crop treatments at each topographical

position ($P < 0.05$). Bold capital letters mark significant differences among marginal means of topographical positions ($P < 0.05$). Italic bold capital letters indicate significant difference of each cover crop treatment across topographical positions ($P < 0.05$).

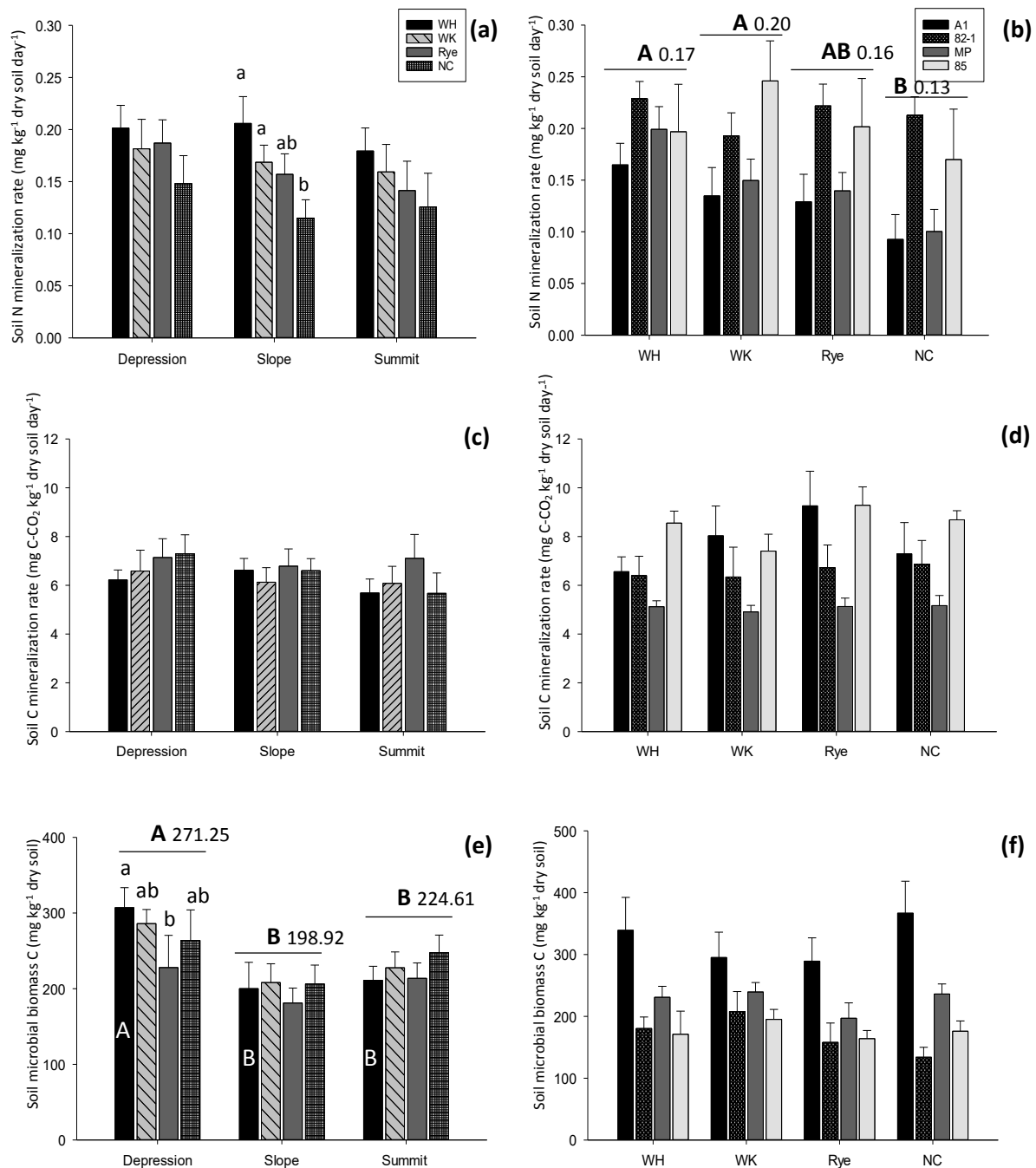


Fig.4. Soil N mineralization (a & b), C mineralization (c & d), and microbial biomass (e & f) at 10 cm depth for the studied cover crop systems and topographical positions across all fields (left side graphs) and at the studied cover crop systems in individual fields across all topographical positions (right side graphs). Shown are means and standard errors. White regular and italic capital letters indicate statistically significant differences among topographical positions within each cover crop treatment ($P < 0.05$). Lowercase letters indicate statistically significant differences among cover crop systems within individual topographical position ($P < 0.05$). Bold capital black letters mark significant differences among marginal means of topographical positions (left side graphs) and of cover crop systems (right side graphs) ($P < 0.05$)

Appendix

Table A1. F test results from statistical analysis for effects of cover crop and topography on soil measured parameters and plant biomass for all studied fields together. Significant values at $P < 0.05$ and $P < 0.1$ were presented in bold and italic bold, respectively. CC = cover crop, Topo = topography.

Variables	All fields together					
	CC		Topo		CC * Topo	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Soil moisture	0.88	0.46	11.67	<0.01	0.72	0.63
Soil NH ₄ ⁺ content	1.01	0.39	0.16	0.85	0.65	0.69
Soil NO ₃ ⁻ content	7.45	<0.01	2.48	0.10	1.09	0.37
Soil N mineralization rate	4.70	<0.01	0.39	0.67	0.25	0.95
Soil C mineralization rate	1.20	0.31	0.45	0.64	0.10	0.36
Soil microbial biomass C	1.97	0.13	2.36	0.11	1.02	0.42
Total spring plant biomass	17.06	<0.01	0.54	0.59	0.46	0.83
Total fall plant biomass	15.20	<0.01	3.22	0.05	1.59	0.15
Aggregate size >2 mm	2.81	0.05	5.25	0.01	1.71	0.12
Aggregate size 0.25-2 mm	4.37	<0.01	4.09	0.03	1.81	0.10
Aggregate size 0.053-0.25 mm	0.39	0.76	2.17	0.13	0.67	0.67

Table A2. F-test results for slicing of the cover crop by topography interactions for soil and plant variables for all studied fields together. Values are presented in bold and italic bold for significant effects at $P < 0.05$ and $P < 0.1$, respectively. CC = cover crop, Topo = topography.

Variables	Effects	Topo	CC	All fields together	
				<i>F</i>	<i>P</i>
Soil moisture	CC * Topo	Depression		0.36	0.78
	CC * Topo	Slope		0.6	0.61
	CC * Topo	Summit		1.38	0.25
	CC * Topo		NC	8.53	<0.01
	CC * Topo		Rye	7.35	<0.01
	CC * Topo		WH	8.37	<0.01
	CC * Topo		WK	11.1	<0.01
Soil NH ₄ ⁺ content	CC * Topo	Depression		0.11	0.95
	CC * Topo	Slope		1.58	0.19
	CC * Topo	Summit		0.32	0.80
	CC * Topo		NC	0.98	0.37
	CC * Topo		Rye	0.04	0.96
	CC * Topo		WH	0.42	0.65
	CC * Topo		WK	0.37	0.69
Soil NO ₃ ⁻ content	CC * Topo	Depression		2.76	0.04
	CC * Topo	Slope		1.35	0.26
	CC * Topo	Summit		5.83	<0.01
	CC * Topo		NC	2.4	0.09
	CC * Topo		Rye	1.9	0.15
	CC * Topo		WH	1.01	0.36
	CC * Topo		WK	3.94	0.02
Soil N mineralization rate	CC * Topo	Depression		1.06	0.36
	CC * Topo	Slope		2.74	0.04
	CC * Topo	Summit		1.29	0.28
	CC * Topo		NC	0.19	0.82
	CC * Topo		Rye	1.00	0.37
	CC * Topo		WH	0.32	0.72
	CC * Topo		WK	0.11	0.89
Soil C mineralization rate	CC * Topo	Depression		0.95	0.42
	CC * Topo	Slope		0.49	0.68
	CC * Topo	Summit		1.18	0.31
	CC * Topo		NC	1.07	0.34
	CC * Topo		Rye	0.65	0.52
	CC * Topo		WH	0.16	0.84
	CC * Topo		WK	0.79	0.45
Soil microbial biomass C	CC * Topo	Depression		3.58	0.01
	CC * Topo	Slope		0.4	0.75
	CC * Topo	Summit		0.73	0.53
	CC * Topo		NC	0.6	0.55
	CC * Topo		Rye	0.14	0.86
	CC * Topo		WH	4.21	0.01
	CC * Topo		WK	1.87	0.15
Total spring plant biomass	CC * Topo	Depression		4.18	<0.01
	CC * Topo	Slope		10.34	<.001
	CC * Topo	Summit		3.90	0.01
	CC * Topo		NC	0.91	0.40
	CC * Topo		Rye	0.52	0.59
	CC * Topo		WH	0.17	0.84
	CC * Topo		WK	0.30	0.74

Total fall plant biomass	CC * Topo	Depression		13.12	<0.001
	CC * Topo	Slope		4.11	0.008
	CC * Topo	Summit		3.28	0.02
	CC * Topo		NC	0.18	0.83
	CC * Topo		Rye	0.00	0.99
	CC * Topo		WH	5.44	0.005
	CC * Topo		WK	3.00	0.05
Aggregate size >2mm	CC * Topo	Depression		5.85	0.001
	CC * Topo	Slope		0.06	0.97
	CC * Topo	Summit		0.20	0.89
	CC * Topo		NC	3.53	0.03
	CC * Topo		Rye	2.20	0.11
	CC * Topo		WH	3.62	0.03
	CC * Topo		WK	3.20	0.04
Aggregate size 0.25-2mm	CC * Topo	Depression		7.07	<0.001
	CC * Topo	Slope		0.10	0.96
	CC * Topo	Summit		0.56	0.63
	CC * Topo		NC	3.42	0.03
	CC * Topo		Rye	1.87	0.15
	CC * Topo		WH	3.19	0.04
	CC * Topo		WK	2.96	0.05
Aggregate size 0.053-0.25mm	CC * Topo	Depression		1.37	0.25
	CC * Topo	Slope		0.22	0.88
	CC * Topo	Summit		0.08	0.96
	CC * Topo		NC	2.45	0.09
	CC * Topo		Rye	0.81	0.44
	CC * Topo		WH	0.50	0.61
	CC * Topo		WK	1.65	0.19

Table A3 Mean values of corn grain yields (Field A1, 82-1, and MP) for individual cover crop treatment at each topographical position

Corn yield (kg/ha)	Depression				Slope				Summit			
	WH	WK	Rye	NC	WH	WK	Rye	NC	WH	WK	Rye	NC
A1	11978 ± 344	13010 ± 411	12215 ± 994	12853 ± 953	10275 ± 301	9983 ± 811	9752 ± 546	10685 ± 423	12690 ± 667	10591 ± 950	10537 ± 564	9885 ± 304
82-1	11663 ± 757	12476 ± 509	11507 ± 1263	11614 ± 1410	11345 ± 1182	12169 ± 678	10703 ± 1264	11541 ± 1013	11751 ± 1042	11534 ± 935	12075 ± 741	10123 ± 564
MP	10032 ± 349	8988 ± 748	9355 ± 325	9175 ± 494	4231 ± 1130	3237 ± 680	3878 ± 978	3725 ± 1175	6612 ± 407	5366 ± 710	5512 ± 657	5421 ± 500

Table A4. Pearson correlation coefficient between soil properties at each topographical position and all topographies together. Bold regular and italic values indicated statistical significance at $P < 0.05$ and $P < 0.1$, respectively.

Variable	Depression			Slope			Summit			All topographies together		
	Aggregate >2mm	Aggregate 0.25-2mm	Aggregate 0.053-0.25 mm	Aggregate >2mm	Aggregate 0.25-2mm	Aggregate 0.053-0.25 mm	Aggregate >2mm	Aggregate 0.25-2mm	Aggregate 0.053-0.25 mm	Aggregate >2mm	Aggregate 0.25-2mm	Aggregate 0.053-0.25 mm
Soil N mineralization rate	-0.191	0.179	0.185	-0.115	0.106	0.138	-0.047	0.083	0.065	-0.081	0.090	0.089
Soil C mineralization rate	-0.109	0.092	0.161	0.110	-0.106	-0.080	-0.066	0.223	-0.081	0.023	0.021	-0.040
Soil microbial C	-0.217	0.210	0.215	0.149	-0.156	-0.105	0.374	-0.423	-0.359	0.067	-0.096	-0.099
Variable	Soil microbial biomass			Soil microbial biomass			Soil microbial biomass			Soil microbial biomass		
Soil N mineralization rate	-0.282			-0.005			-0.283			-0.151		
Soil C mineralization rate	-0.134			-0.142			-0.186			-0.130		

Table A5. F test results from statistical analysis for effects of cover crop and topography on soil measured parameters and plant biomass for individual studied field. Significant values at $P < 0.05$ and $P < 0.1$ were presented in bold and italic bold, respectively. CC = cover crop, Topo = topography.

Variables	Field A1						Field 82-1						Field MP						Field 85					
	CC		Topo		CC * Topo		CC		Topo		CC * Topo		CC		Topo		CC * Topo		CC		Topo		CC * Topo	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Soil moisture	2.03	0.17	1.02	0.39	0.74	0.62	0.26	0.85	4.94	0.05	0.45	0.82	1.58	0.20	12.02	0.03	0.43	0.85	1.23	0.37	7.40	0.02	1.78	0.21
Soil NH ₄ ⁺ content	0.92	0.47	0.44	0.65	1.21	0.34	0.78	0.53	0.13	0.87	1.36	0.33	0.60	0.61	1.19	0.41	0.46	0.83	0.62	0.62	0.97	0.43	0.39	0.86
Soil NO ₃ ⁻ content	4.17	0.04	1.64	0.24	0.61	0.71	1.68	0.24	1.06	0.40	2.64	0.10	3.2	0.02	8.99	0.05	1.49	0.19	4.67	0.05	4.22	0.07	2.06	0.15
Soil N mineralization	2.11	0.16	1.07	0.38	0.63	0.70	1.03	0.42	2.61	0.15	3.16	0.06	4.2	0.01	2.57	0.22	0.35	0.91	0.22	0.88	0.25	0.78	0.76	0.61
Soil C mineralization	1.38	0.31	0.22	0.81	0.67	0.67	0.32	0.81	1.30	0.34	1.23	0.38	0.21	0.88	16.87	0.02	1.62	0.15	2.00	0.21	1.07	0.40	1.61	0.25
Soil microbial biomass C	1.14	0.38	0.57	0.58	0.52	0.78	5.53	0.01	0.22	0.81	4.1	0.01	1.32	0.27	3.87	0.14	1.66	0.14	0.97	0.46	3.17	0.11	2.51	0.11
Total spring plant biomass	24.15	<0.01	1.41	0.29	6.83	0.01	4.66	0.03	0.13	0.88	1.15	0.41	5.05	<0.01	1.58	0.33	0.65	0.68	18.98	<0.01	0.26	0.77	0.69	0.66
Total fall plant biomass	2.69	0.11	0.42	0.67	1.68	0.22	2.08	0.19	0.26	0.77	0.30	0.88	19.2	<0.001	3.30	0.17	2.23	0.05	4.81	0.04	0.83	0.47	1.91	1.17
Aggregate size >2mm	0.22	0.87	3.62	0.07	2.33	0.09	1.51	0.27	3.85	0.08	1.53	0.29	4.69	<0.01	5.92	0.09	1.25	0.29	-	-	-	-	-	-
Aggregate size 0.25-2mm	0.38	0.76	4.24	0.05	2.70	0.06	0.89	0.48	5.80	0.03	0.83	0.58	5.55	<0.01	1.87	0.29	0.69	0.65	-	-	-	-	-	-
Aggregate size 0.053-0.25mm	0.65	0.60	0.80	0.47	1.40	0.29	1.64	0.24	4.27	0.07	0.82	0.58	1.53	0.21	5.25	0.10	1.32	0.26	-	-	-	-	-	-

Table A6. F-test results for slicing of the cover crop by topography interactions for soil and plant variables for individual studied fields. Values are presented in bold and italic bold for significant effects at $P < 0.05$ and $P < 0.1$, respectively. CC = cover crop, Topo = topography.

Variables	Effect	Topo	CC	Field A1		Field 82-1		Field MP		Field 85	
				<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Soil moisture	CC*Topo	Depression		1.85	0.17	0.20	0.89	0.98	0.40	4.07	0.04
	CC*Topo	Slope		0.87	0.47	0.82	0.51	0.12	0.94	0.4	0.75
	CC*Topo	Summit		1.05	0.39	0.18	0.90	1.39	0.25	0.08	0.96
	CC*Topo		NC	0.75	0.48	2.44	0.14	6.74	<0.01	1.78	0.22
	CC*Topo		Rye	0.41	0.67	3.96	0.06	1.97	0.14	5.43	0.02
	CC*Topo		WH	0.77	0.47	3.96	0.06	3.56	0.03	7.58	0.01
	CC*Topo		WK	2.01	0.16	2.77	0.12	3.04	0.05	10.72	<0.01
Soil NH ₄ ⁺ content	CC*Topo	Depression		0.08	0.96	0.72	0.56	0.16	0.92	0.17	0.91
	CC*Topo	Slope		3.18	0.04	0.22	0.87	0.17	0.91	1.25	0.34
	CC*Topo	Summit		0.02	0.99	2.14	0.17	1.17	0.32	0.12	0.94
	CC*Topo		NC	3.75	0.04	0.98	0.41	0.67	0.51	0.26	0.77
	CC*Topo		Rye	0.02	0.99	0.04	0.96	0.33	0.71	1.6	0.25
	CC*Topo		WH	3.75	0.04	0.21	0.81	0.93	0.39	0.08	0.92
	CC*Topo		WK	0.25	0.78	2.98	0.10	0.61	0.54	0.12	0.88
Soil NO ₃ ⁻ content	CC*Topo	Depression		1.63	0.21	0.44	0.72	0.19	0.90	5.42	0.02
	CC*Topo	Slope		3.51	0.03	0.67	0.59	0.74	0.53	0.41	0.75
	CC*Topo	Summit		0.25	0.86	5.57	0.02	5.13	<0.01	3.75	0.05
	CC*Topo		NC	1.11	0.35	1.14	0.36	6.60	<0.01	2.55	0.13
	CC*Topo		Rye	2.29	0.13	1.03	0.39	4.12	0.02	1.17	0.35
	CC*Topo		WH	0.64	0.54	1.19	0.35	3.95	0.02	0.39	0.68
	CC*Topo		WK	1.37	0.27	4.74	0.04	2.29	0.11	6.24	0.01
Soil N mineralization rate	CC*Topo	Depression		0.93	0.44	0.14	0.93	0.69	0.55	0.45	0.72
	CC*Topo	Slope		2.21	0.12	1.1	0.40	1.60	0.19	0.12	0.94
	CC*Topo	Summit		0.23	0.87	5.57	0.02	2.54	0.06	0.73	0.55
	CC*Topo		NC	0.17	0.84	2.51	0.14	1.66	0.19	0.33	0.72
	CC*Topo		Rye	1.26	0.31	4.3	0.05	2.10	0.12	0.88	0.44
	CC*Topo		WH	1.26	0.31	0.96	0.42	0.22	0.80	0.42	0.66
	CC*Topo		WK	0.72	0.50	4.13	0.05	0.23	0.79	0.75	0.49
Soil C mineralization rate	CC*Topo	Depression		0.90	0.45	1.08	0.40	1.61	0.19	0.15	0.92
	CC*Topo	Slope		0.40	0.75	0.51	0.68	1.15	0.33	4.47	0.04
	CC*Topo	Summit		1.42	0.25	0.28	0.83	0.67	0.57	0.58	0.64
	CC*Topo		NC	0.13	0.87	1.55	0.27	8.19	<0.01	0.08	0.92
	CC*Topo		Rye	0.45	0.64	2.41	0.15	7.25	<0.01	0.12	0.88
	CC*Topo		WH	0.27	0.76	0.47	0.63	0.81	0.44	1.04	0.39
	CC*Topo		WK	0.87	0.43	0.11	0.89	5.55	<0.01	4.56	0.04
Soil microbial biomass C	CC*Topo	Depression		1.58	0.22	8.47	<0.01	2.2	0.09	3.08	0.09
	CC*Topo	Slope		0.46	0.71	1.29	0.34	0.63	0.59	2.04	0.18
	CC*Topo	Summit		0.15	0.93	1.77	0.23	1.82	0.14	0.88	0.49
	CC*Topo		NC	1.30	0.29	0.75	0.50	2.86	0.06	0.43	0.66
	CC*Topo		Rye	0.11	0.89	5.85	0.02	2.39	0.09	1.63	0.25
	CC*Topo		WH	0.41	0.66	1.78	0.22	2.88	0.06	8.43	0.01
	CC*Topo		WK	0.40	0.67	6.13	0.02	2.54	0.08	2.1	0.18
Total spring plant biomass	CC*Topo	Depression		10.51	<0.01	2.08	0.18	0.61	0.61	6.86	0.01
	CC*Topo	Slope		30.55	<0.01	1.99	0.19	4.16	<0.01	3.85	0.05
	CC*Topo	Summit		3.34	0.08	2.71	0.11	1.6	0.19	9.91	<0.01
	CC*Topo		NC	0.13	0.87	0.76	0.50	1.9	0.15	0.02	0.98
	CC*Topo		Rye	0.10	0.91	0.26	0.77	0.85	0.42	0.29	0.75
	CC*Topo		WH	17.21	<0.01	1.06	0.38	0.34	0.71	1.05	0.38
	CC*Topo		WK	0.05	0.94	0.72	0.51	2.05	0.13	0.98	0.41

Variable			Effect	Topo	CC	Field A1		Field 82-1		Field MP		Field 85	
						<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Total fall plant biomass			CC*Topo	Depression		3.29	0.06	0.93	0.62	16.5	<0.001	2.44	0.12
			CC*Topo	Slope		1.38	0.30	1.03	0.60	1.38	0.25	3.48	0.05
			CC*Topo	Summit		1.56	0.26	0.82	0.65	6.63	0.0005	5.58	0.01
			CC*Topo		NC	0.07	0.93	0.15	0.87	0.14	0.87	0.94	0.42
			CC*Topo		Rye	0.01	0.98	0.33	0.77	0.01	0.91	0.12	0.88
			CC*Topo		WH	3.14	0.08	0.30	0.79	8.98	0.0001	2.44	0.13
			CC*Topo		WK	2.50	0.13	0.34	0.77	2.06	0.13	2.75	0.11
Aggregate >2mm	size		CC*Topo	Depression		1.92	0.17	2.94	0.11	4.63	<0.01		
			CC*Topo	Slope		1.91	0.17	0.75	0.55	0.98	0.41		
			CC*Topo	Summit		1.07	0.39	0.62	0.62	1.68	0.17		
			CC*Topo		NC	3.26	0.07	1.53	0.28	0.42	0.66		
			CC*Topo		Rye	0.48	0.62	6.56	0.02	1.74	0.18		
			CC*Topo		WH	5.85	0.01	3.27	0.09	2.22	0.11		
			CC*Topo		WK	0.43	0.65	1.37	0.31	5.34	<0.01		
Aggregate 0.25-2mm	size		CC*Topo	Depression		2.81	0.08	1.23	0.36	4.47	<0.01		
			CC*Topo	Slope		2.38	0.11	0.84	0.51	1.08	0.36		
			CC*Topo	Summit		0.83	0.50	0.46	0.71	1.40	0.24		
			CC*Topo		NC	3.20	0.07	3.06	0.10	0.21	0.81		
			CC*Topo		Rye	0.97	0.40	6.88	0.02	0.66	0.52		
			CC*Topo		WH	7.28	<0.01	2.82	0.11	1.26	0.28		
			CC*Topo		WK	0.55	0.59	2.52	0.14	1.86	0.16		
Aggregate 0.053-0.25 mm	size		CC*Topo	Depression		0.57	0.64	1.89	0.21	1.94	0.12		
			CC*Topo	Slope		1.34	0.31	0.95	0.46	1.04	0.38		
			CC*Topo	Summit		1.22	0.34	0.54	0.66	1.20	0.31		
			CC*Topo		NC	1.53	0.25	0.70	0.52	0.82	0.44		
			CC*Topo		Rye	0.03	0.97	4.21	0.06	1.99	0.14		
			CC*Topo		WH	2.16	0.15	4.86	0.04	1.00	0.37		
			CC*Topo		WK	0.64	0.54	1.26	0.33	5.31	<0.01		

Table A7 The C/N ratio of cover crops

Cover crops	C:N
Cereal rye	18
WK oat	16
WK winter pea	11
WK radish	10
WH ryegrass	26
WH rapeseed	17
WH crimson clover	13



Fig A1. Studied field layout in Kellogg Biological Station (KBS): field A1, field 82-1, field MP, and field 85 showing positions of individual field, topographic positions, and treatment blocks. The blue boxes indicate blocks and yellow dash boxes indicate topographic positions

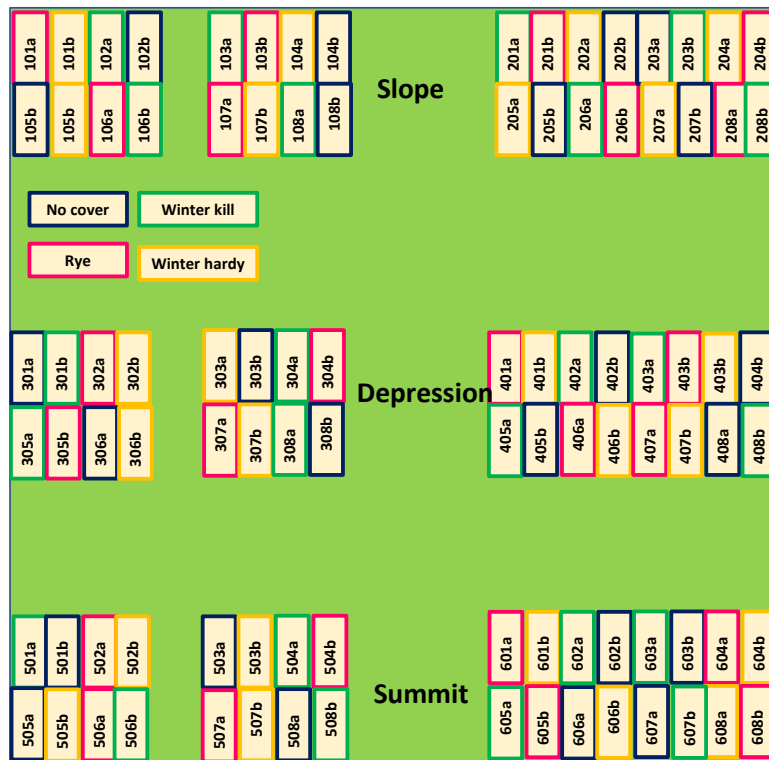


Fig A2. The field layout of MP field in KBS which consisted of two blocks located within each topographical position with 8 experimental plots (28.18 by 31.92 ft) within each block, randomly assigned to cover crop treatments.

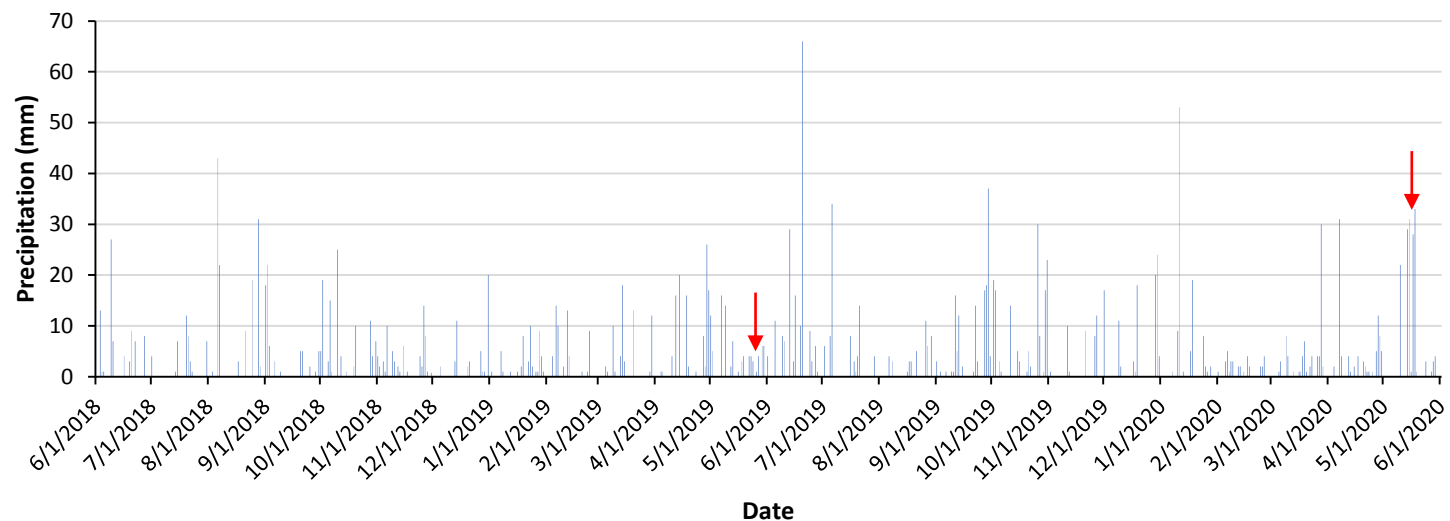


Fig A3. Precipitation at KBS during the first year of organic transition (2018-2019 for fields A1, 82-1 and MP; 2019-2020 for field 85). Red arrows indicate spring sampling dates in 2019 for fields A1, 82-1 and MP; and in 2020 for field 85.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

1 Soil properties after one year of interseeded cover cropping in topographically diverse
2 agricultural landscape

3 Linh T.T. Nguyen, Kaleb A. Ortner, Lisa K. Tiemann, Karen A. Renner, Alexandra N.
4 Kravchenko

5
6 Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing MI
7 48824, United States

Abstract

Planting cover crops within or following a cash crop may improve soil-based ecosystem services due to increased plant diversity and a longer duration of live vegetation coverage. We examined the effect of three different cover cropping systems on soil properties after one year of a three-year organic transition rotation at four agricultural field sites with contrasting topographical positions, namely depressions, slopes, and summits. The four studied systems were (1) cereal rye (*Secale cereal* L.) planted after corn (*Zea mays* L.) harvest (Rye); (2) a mixture of cold susceptible cover crop species, namely, oat (*Avena sativa*), winter pea (*Pisum sativum*), and radish (*Raphanus sativus*), interseeded into corn (WK); (3) a mixture of cold tolerant cover crop species, namely, annual ryegrass (*Lolium multiflorum*), Dwarf Essex rapeseed (*Brassica napus*), and crimson clover (*Trifolium incarnatum*), interseeded into corn (WH); and (4) a no-cover control (NC). While soil moisture was affected by topography, interseeding cover crops into corn did not influence soil moisture levels at the 0-10 cm depth for the studied year. Soil NO₃⁻ content was markedly higher in the WK system compared to cereal rye and WH cover crop treatments. The difference was especially pronounced in depressions and summits. Soil N mineralization rates followed the pattern WH>WK>Rye>NC and the effects were most pronounced in slopes. Soil microbial biomass C was the highest in depressions followed by summits and slopes, and in depressions the WH had higher microbial biomass than the other systems. There were no effects of cover crops and topography on soil C mineralization one year after the organic transition was initiated. The WH system increased the fraction of 0.053-2 mm aggregates and decreased > 2 mm aggregates in depressions. The results suggest that the effect of cover cropping can become evident already one year after the organic transition begins. The WH mixture interseeded into the cash crop was an optimal cover crop choice for improving soil characteristics as well as decreasing soil N leaching risks during

organic transition in undulating agricultural terrain. However, the magnitude of the benefit provided by WH was mediated by topography.

Keywords: Organic transition, Cover crop mixtures, Plant diversity, Interseeding in corn, Soil based ecosystem services, Topography, Maize.

1. Introduction

Organic agriculture provides vital environmental benefits, including improvements in soil fertility, enhancement in biodiversity, and mitigation of groundwater pollution and global warming (Mondelaers et al., 2009; Tuomisto et al., 2012; Gattinger et al., 2012; Hubbard et al., 2013; Smith et al., 2019). With rising prices of organic products and concerns over environmental sustainability, agricultural producers have become increasingly interested in developing organic farming systems. However, obtaining an official organic certification requires a 36-month transition period (Webber et al., 2009). During this time, all synthetic inputs including chemical fertilizers and pesticides are prohibited which may lead to crop yield losses and reduced profits (Delbridge et al., 2017). Since cover cropping can improve soil quality and fertility (Mitchell et al., 2017; Ghimire et al., 2019), one of the strategies to overcome the potential yield loss during organic transition is including cover crops in the rotation (Kim et al., 2020).

Cover crops improve soil quality by strengthening soil structure, reducing soil erosion and nutrient loss, increasing soil carbon (C) and nitrogen (N) stocks, improving drainage, enhancing soil microbial communities, and suppressing weeds (Blanco-Canqui et al., 2015; Vukicevich et al., 2016; Schmidt et al., 2018; Hill et al., 2016; Nichols et al., 2020). In the US Midwest, cover crop-based organic transition in row crop rotation systems, particularly corn (*Zea mays*)-soybean (*Glycine max*)-winter wheat (*Triticum aestivum*) rotation (CSW), has gained popularity due to its feasibility and low financial risks (Silva and Delate, 2017). However, cover-cropping can be challenging due to increased management demands, cover crop establishment issues, and cover crop species selection uncertainties (Plastina et al., 2018). Among the management issues frequently faced by farmers of the US Midwest are insufficient time for post-harvest cover crop planting in the fall and time-shortages for pre-plant cover crop termination in the spring. The former issue is exacerbated when weather conditions force

1 relatively late (October-November) row crop, e.g., corn, harvesting, leaving a very short
2 window for fall cover crop establishment. The latter issue can be particularly troubling during
3 wet and cold springs when suitable soil conditions for timely tillage and planting can be hard
4 to come-by; the concern is aggravated by climate-change-induced increases in extreme
5 precipitation in the spring (Byun and Hamlet, 2018). This can substantially limit the cover crop
6 species options available to farmers and lead to poor establishment and growth of cover crops
7 (Midwest Cover Crops Council, 2015).

8 One potential approach to maximize the benefits of cover cropping is to increase cover
9 crop diversity (Robertson et al., 2014; Vukicevich et al., 2016) by using mixtures of several
10 plant species (Tribouillois et al., 2016). A recent meta-analysis for 27 studies which compared
11 cover crop mixtures and their constituent species reported that 88% of the comparisons were
12 similar in biomass, N retention and weed suppression (Florence and McGuire, 2020). Also,
13 10% of the comparisons showed better performance of monocultures while 2% of the
14 comparisons indicated that cover crop mixtures did better (Florence and McGuire, 2020). There
15 has been existing evidence that cover crop mixtures are more productive than component
16 monocultures in producing biomass (Smith et al., 2014; Finney et al., 2016). Such increases in
17 plant biomass are likely due to complementary contributions of species with different patterns
18 in root architecture, plant physiology and phenology (Hooper et al., 2005). For example, grass-
19 legume cover crop mixtures often produce biomass either equal to or greater than single species
20 (Ranells and Waggoner, 1996; Hayden et al., 2014) suggesting that combining cover crops with
21 different N functionality may lead to increased total plant biomass. Additionally, cover crop
22 mixtures of legume and non-legume species can improve soil N retention and the release of N
23 to subsequent crops (Hayden et al., 2014; White et al., 2017). Given the potentially
24 multifunctional advantages derived from diverse cover crop mixtures, their use can optimize
25 ecosystem services and maintain crop productivity during the organic transition.

1 However, it is difficult to find multiple species that will establish late in the fall. A
2 practice of interseeding, that is planting a cover crop or a cover crop mixture into a growing
3 cash crop, can overcome this difficulty (Donaldson et al., 2019; Mohammed et al., 2020;
4 Sullivan et al., 2020). Interseeding of cover crop mixtures extends the duration of soil coverage
5 by a diverse plant community without, under best management practice, jeopardizing main
6 crop growth and development (Belfry and Van Eerd, 2016; Curran et al., 2018; Brooker et al.,
7 2020a; Rusch et al., 2020). Interseeded cover crops can scavenge excess nitrogen (N), thereby
8 reducing potential nutrient leaching and runoff (Mohammed et al., 2020), and can decrease
9 weed pressure. Youngerman et al. (2018) indicated a 31% weed biomass reduction by
10 interseeding cover crops into organic corn. Mixtures of legumes, grasses, and *Brassica* spp.
11 increased soil water content and soil inorganic N compared to single and double species of
12 cover cropping in a 3-year study of corn-soybean systems (Chu et al., 2017).

13 Topography can substantially impact soil properties and plant performance (Munoz et
14 al., 2014), including cover crop performance and the benefits they generate (Ladoni et al.,
15 2015). Ladoni et al. (2015) reported that red clover cover crop increased soil NO₃⁻ contents by
16 35% in depressions, 20% on slopes, and 32% on summits, while cereal rye significantly
17 decreased soil NO₃⁻ content in depressions, but not on slopes and summits. Beehler et al. (2017)
18 observed higher soil particulate organic C under cover crop treatments as compared to controls
19 only on summits and slopes, but not in topographical depressions. Munoz et al. (2014) reported
20 interactive effects of cover crops and topographical positions on cash crops in corn-soybean-
21 wheat rotation systems. Red clover positively influences corn yields, but the magnitude was
22 more pronounced in topographical slopes and summits (Munoz et al., 2014). Even though
23 commercial agricultural fields are often large and topographically diverse, the role of
24 topography in mediating cover crop effects on soil and plant performances is poorly
25 understood. Such understanding is necessary for the development of effective management

practices in organic transition to maximize benefits derived from cover crops in undulating agricultural terrain.

Here, we examine three cover crop systems implemented during the first year of the three-year CSW organic transition rotation and compare them to the no cover control (NC). The three systems together with the no-cover control represent a gradient of plant diversity and green canopy cover duration. In the spring of 2019 and 2020 we collected data from 4 experimental sites that began the organic transition one year prior. All fields had diverse topography representative of the undulating agricultural landscapes of the US Midwest: depressions, representing footslope and toeslope positions; slopes, representing backslope and shoulder positions; and summits, representing summit positions.

2. Materials and Methods

2.1. Field site description

The study was carried out in 4 experimental fields at Kellogg Biological Station (KBS) (42° 24' N, 85° 24' W), Michigan (Appendix Fig.A1). The region's mean annual temperature is 10.1°C and annual rainfall is 1005 mm with about half received as snow (Robertson and Hamilton, 2015). KBS soils are well-drained Typic Hapludalfs of Kalamazoo (fine loamy, mixed, mesic) and Oshtemo (coarse loamy, mixed, mesic) series, developed on glacial washout from the last Wisconsin glaciation (Crum and Collins, 1995). Baseline data for studied fields are presented in Table 1. Soil samples for baseline analysis were collected prior to the experiment initiation at a depth of 0-20 cm.

2.2. Experimental design

In three fields namely fields A1, 82-1, and 85, the experiment was set up as a split-block design with two main factors: topographical position with three levels: depression, slope,

and summit, and the type of cover crop with four levels: no cover control; traditional cereal rye (Rye) planted after corn harvest; a cold susceptible mixture of oat, winter pea, and radish, interseeded into corn at the V5-V6 developmental stage and subsequently terminated by killing frosts (WK, short for winter kill); and a cold resistant mixture of ryegrass, rapeseed, and clover, interseeded into corn at the V5-V6 growth stage and terminated prior to spring soybean planting (WH, short for winter-hardy). The three-species WK and WH mixtures can be difficult to establish in late fall, yet their interseeding offers a substantially longer period of active growth. Interseeding of WH and WK systems addresses the issue of insufficient time for post-harvest cover crop planting, while WK system addresses the issue of time shortage for pre-plant cover crop termination in spring. The WH system has high plant diversity and the longest green plant coverage lasting through fall and spring preceding the main crop planting; WK is the high diversity system with green coverage only during the preceding fall; and cereal rye is the low diversity system with effective green coverage in late fall and the spring prior to main crop planting

Two fields (A1 and 82-1) were divided into 4 replicated blocks, and one field (85) was divided into 3 replicated blocks, with blocks placed across all topographical positions. Each block was divided into 4 experimental plots (4.5 m in width and ranging from 19 to 372 m in length depending on the field size). The cover crop treatments were assigned at random to the plots within each block. The fourth field, MP, consisted of two blocks located within each topographical position with 4 experimental plots (8.5 by 19.5 m within each block, randomly assigned to cover crop treatments (Appendix Fig. A2).

2.3. Field and crop management during organic transition

Organic transition requires a period of 36 months prior to the start of the certified organic production. This study focused on the first year of 36 months organic transition CSWR which

lasted from May 2018 to June 2019 for A-1, 82-1, and MP fields, and from May 2019 to June 2020 for field 85.

Corn (*Zea mays* L. cv. Pioneer variety P0414AM) was planted in 76 cm rows at a seeding rate of 69,000 seeds ha⁻¹ in May 2018 in fields A-1, 82-1, and MP, and in May 2019 in field 85 (Appendix, Fig.A1). Before planting, mineral fertilizers including potash and monoammonium phosphate were applied at a rate of 180 kg ha⁻¹ and 113 kg ha⁻¹, respectively. Also, dolomitic lime was spread across the fields at a rate of 3400 kg ha⁻¹. A soil finishing tool was then used to prepare seed bed for planting corn. Prior to the organic transition experiment, field A1 and 82-1 were planted to wheat, corn was grown on field MP, while field 85 was soybean with a wheat cover crop.

Two cover crop mixtures, WK and WH, were interseeded by broadcasting onto the fields A1, 82-1 and MP (Appendix, Fig A2) when corn was at the V5-V6 developmental stage. For field 85, the WH and WK cover crop mixtures were interseeded in the V5-V6 corn with an interseeder with drop tubes to spread seed between the corn rows. Specifically, in June 2018 (the fields A1, 82-1, and MP) and June 2019 (the field 85), the WK mixture of oat (*Avena sativa* L. cv. Jerry), winter pea (*Pisum sativum* L. cv. Windham winter pea), and radish (*Raphanus sativus* L. cv. Nitro radish) was seeded at 28, 23 and 2 kg ha⁻¹ respectively. The WH mixture of annual ryegrass (*Lolium multiflorum* L. cv. KB Royal annual ryegrass), Dwarf Essex rapeseed (*Brassica napus* L. cv. Dwarf Essex Rape) and crimson clover (*Trifolium incarnatum* L. cv. Dixie crimson clover) was seeded at 9, 2 and 5 kg ha⁻¹. Corn was harvested in November 2018 (fields A1, 82-1, and MP) and November 2019 (field 85), followed by cereal rye (*Secale cereal* L. cv. VNS cereal rye) planting in the Rye cover crop system at a seeding rate of 125 kg ha⁻¹ for the traditional cover crop system. The cover crop seeding rates in this study were recommended rates typical in the Midwest (Fisher et al., 2012).

The fields were chisel-plowed at the end of May the next year to terminate cereal rye and the WH cover crops. Soybean was then planted in 76 cm rows at a seeding rate of 450,000 seeds ha⁻¹ on June 3, 2019 for the fields A1, 82-1, and MP and in June 2, 2020 for field 85.

2.4. Soil sampling and physical and chemical property measurements

Soil samples at a depth of 10 cm were collected in spring after the first year of transition, prior to soybean planting. Sampling took place on May 24, 2019 (2 days before chisel plowing) in fields A1, 82-1, and MP and on May 15, 2020 in field 85. Two soil cores (5 cm diameter x 10 cm in depth) were collected within each topographical position of each plot from fields A1, 82-1, and MP. Four push-probe soil cores (2 cm diameter and 10 cm in depth) were collected from each topographical position of each plot from field 85. Soil samples were passed through 4 mm sieve to remove plant residues and stones and then kept at 4°C prior to analyses. Soil gravimetric water content was determined immediately after field sampling by oven-drying 10 g sub-samples at 104°C for 24h. Soil total C and N were measured using a LECO macro-CN analyser after oven-drying 10 g of sub-samples at 40°C for 72 h and subsequently grinding them to a fine powder. Soil inorganic N including NO₃⁻ and NH₄⁺ was extracted with 2M potassium chloride and then filtered by Whatman no.1 filter paper prior to measurement using a micro plate method (Keeney and Nelson, 1982; Doane and Horwath, 2003). In brief, to 100µl of each sample we added reagents (salicylate and cyanurate for NH₄⁺ and vanadium (III), sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride for NO₃⁻) to form colorimetric solutions. We measured absorbance of these solutions to determine NH₄⁺ and NO₃⁻ on a Synergy H1 spectrophotometer (Biotek, Vermont, USA) at wavelengths of 630 nm and 540 nm respectively. Absorbance values were converted to NH₄⁺ and NO₃⁻ concentrations using standard curves formulated from a series of known concentration of (NH₄)₂SO₄ and KNO₃, respectively.

2.5. Soil dry aggregate fractionation

Soil aggregate fractionation was conducted following the method described by Tiemann and Grandy (2015). Briefly, 100 g soil from each sample was spread on a large weigh boat and subsequently air-dried for several days until its moisture dropped to 10% for dry sieving (Kristiansen et al., 2006). Dry sieving was conducted on a rotary shaker (Retsch AS 200) using three sieves to obtain three aggregate size fractions: 0.053-0.25 mm, 0.25-2 mm, > 2mm. Sieving was conducted for 2 min and in three replicates. Dry aggregate size values from three replicates were averaged and expressed as a unit of g kg⁻¹ dry soils.

2.6. Short-term C mineralization and potential N mineralization rates

Short-term mineralizable C was measured for all collected samples following the procedures described by Franzluebbers et al. (2000) and Culman et al. (2013). Specifically, 10 g of each soil sample was weighed into a 50 mL beaker and was subsequently added an appropriate amount of milli-Q water to bring up the soil moisture to 60% of field capacity. The beaker was subsequently placed into a Mason jar. Water with a volume of 15 mL was added to the bottom of the jar to maintain humidity. The jars were sealed with accompanying lids, where each lid had two holes drilled in the center and septa placed in the holes to accommodate gas sampling. The jars were pre-incubated in the dark at room temperature (about 22°C) for 5 days. After pre-incubation, the jars were kept opened for at least 5 minutes to remove produced CO₂ gases. The lids were then closed, and the jars were subjected to a 10-day incubation to measure soil C mineralization. Soil CO₂ emission was determined by using infrared Photoacoustic Spectroscopy (PAS) (1412 Photoacoustic multi-gas monitors; INNOVA Air Tech Instruments, Ballerup, Denmark).

Soil N mineralization rate was determined as the net amount of NO_3^- and NH_4^+ produced in the soil incubated at field capacity and room temperature (about 22°C) over a period of 28 days (Hart et al., 1994). Briefly, 10 g of each soil sample was placed into a 50 mL Falcon tube which was loosely closed with a cap. Soil moisture content was then adjusted to 60% field capacity by adding milli-Q water. Soil inorganic N content before and after the incubation was extracted using 2M KCl and subsequently filtered through Whatman no.1. Soil NH_4^+ and NO_3^- concentrations (ppm) were measured by the microplate method as described in section 2.4. The rate of N mineralization was determined as the difference in NO_3^- and NH_4^+ content between pre- and post-incubated samples.

2.7. Soil microbial biomass carbon

Soil microbial biomass C was measured using fumigation-incubation method (Jenkinson and Powlson 1976). Control and samples for fumigation were prepared 5-day pre-incubation in dark at room temperature as described in section 2.5. Samples were subsequently transferred to a vacuum desiccator for chloroform fumigation for 24h. Fumigated samples were then removed from the desiccator and then placed into Mason jars for 10-day incubation at room temperature. After incubation, production of CO_2 gas from control and fumigated samples was measured by using infrared Photoacoustic Spectroscopy (PAS) (1412 Photoacoustic multi-gas monitor; INNOVA Air Tech Instruments, Ballerup, Denmark). Microbial C biomass was obtained from the following formula:

$$\text{MBC} = F_c / K_c \text{ (Jenkinson and Powlson 1976; Paul, 2007)}$$

where, MBC is microbial biomass, F_c is equal to [($\text{CO}_2\text{-C}$ evolved from fumigated soil during the 10-day incubation) – ($\text{CO}_2\text{-C}$ evolved from the control during the 10-day incubation)] and K_c , fraction of biomass C mineralized to CO_2 , value of 0.41 was used. K_c is a constant representative of cell utilization efficiency of the fumigation procedure (Paul, 2007).

2.8. Aboveground biomass and plant C and N content measurement and corn yields

Cover crop and weed biomass were collected in November (fall) 2018 and May (spring) 2019 for the field A1, 82-1 and MP. For field 85, cover crop and weed biomass were collected in November (fall) 2019 and May (spring) 2020. Plants were collected separately by species from two quadrats (50 x 50 cm) randomly placed within each plot per topographical position, but, ensuring that the quadrats were approximately 1.5 m away from plot edges to avoid border effects (Wortman et al., 2012). Cover crop and weed biomass were then determined after oven-drying at 60°C for at least one week.

Plant C and N contents were measured via combustion analysis using a Costech ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Inc., Valencia, California).

Corn grain was harvested from two center rows of each plot by using a plot combine. The weight of harvested grain was recorded for corn yields (Brooker et al., 2020a).

2.9. Statistical analysis

Effects of cover crop, topography, and their interactions were examined using mixed effect model in SAS 9.4 (SAS Institute, Cary, NC, USA). The statistical model consisted of the fixed effects of cover crop, topography, and their interaction. For individual field analyses in fields A1, 82-1, and 85, the statistical model consisted of blocks, interaction between blocks and cover crops, and interaction between blocks and topography as random effects, where the latter two were used as error terms for testing the statistical significance of the main effects of cover crops and topography, respectively. For field MP, the interaction between blocks and topography was used as an error term for testing the topography effect. For the analyses of all fields together, fields were added to the model as a random factor, and all the previously described random effects were specified as nested within their respective fields. Since assessing

the interactions between topography and cover crop was the main objective of the study, we reported the means of each cover crop system separately for each topographical position, and sliced the interaction terms by conducting pre-planned simple F-tests for all studied variables. When the slicing F-tests were statistically significant we conducted multiple comparisons using t-tests. When the main effects of the cover crop systems or topographical positions were statistically significant, we compared the marginal means using t-tests. Results at $P < 0.05$ were reported as statistically significant and at $P < 0.1$ as marginally significant or trends.

Pearson correlation analyses were performed to assess relationships between measured variables including soil physicochemical properties (soil moisture, total C and N, soil inorganic N, and aggregate size), soil process rates (soil respiration and N mineralization), and plant biomass (cover crop and weed). The analyses were performed using SPSS software version 26.0 (IBM, Armonk, NY, USA).

The assumptions of normality and equal variances were checked using normal probability plots of the residuals and plots of the residuals vs. predicted values, respectively. We did not detect assumption violations.

3. Results

3.1. Soil physicochemical properties

Generally, soil moisture at the sampling time was the lowest in slopes, followed by summits, and then depressions for all cover crop treatments (Fig.1a, Appendix Table A1, $P < 0.01$). Cover crops did not influence soil moisture (Fig.1b, $P = 0.46$).

Soil NH_4^+ content was not affected by either cover crop or topography (Fig.1c & d, Appendix Table A1, $P > 0.05$). In contrast, soil NO_3^- contents of the cereal rye and WH cover crop systems were $\sim 25\%$ lower than those of the control and WK treatments (Fig.1e & f, Appendix Table A1, $P < 0.05$), with the differences among the cover crops most pronounced

in summits and depressions (Fig. 1c). Soil NO_3^- content in slopes was 25-30% lower than that in summits and depressions (Fig. 1e, $P < 0.05$). The differences between topographical positions were particularly pronounced in the WK and NC systems (Fig. 1e). Soil NO_3^- content of the WK treatment in slopes was 32-37% lower than that in summits and depressions, respectively (Appendix Table A2, $P = 0.02$) while soil NO_3^- content of the control in summits was about 25% higher than that of the NC treatment in slopes and depressions (Appendix Table A2, $P = 0.09$).

The >2 mm aggregate fraction was the lowest in the WH system, while the 0.25-2 mm fraction was the highest in the WH system; both trends were most pronounced in depressions (Fig. 2a & c). The >2 mm aggregate fraction in summits was significantly lower than that in depressions and slopes (Fig. 2a, $P = 0.01$) whereas the 0.053-2 mm fraction in summits was significantly higher than that in depressions and slopes (Fig. 2c, $P = 0.03$). However, it should be noted that the effects of topography varied among the systems. In the WH system, unlike in the other three systems, the >2 mm aggregate fraction was higher in slopes than in depressions and summits and the 0.25-2 mm fraction was as high in depressions as in summits (Fig. 2a & c). Cover crop systems did not differ in terms of 0.053-0.25 mm aggregate fraction contents (Fig. 2e & f). Summits had significantly higher amounts of this fraction as compared to depressions, the difference most pronounced in the NC control (Fig. 2e, $P < 0.05$).

3.2 Total aboveground plant biomass and corn yields

In the fall of the first year of the organic transition, total plant biomass was the highest in depressions, followed by summits and then slopes (Fig. 3a, Appendix Table A1, $P = 0.05$). The WH system produced significantly higher biomass than the WK system in depressions and slopes (Fig. 3a, $P < 0.05$) whereas there was no significant difference in biomass between the WH and WK treatments in summits (Fig. 3a, $P > 0.05$). The WH in depressions produced

1 clover biomass was 3.6 fold higher in depressions compared with summits (Fig.3a). In contrast,
2 pea biomass was 8 fold higher in the summit compared with the depression (Fig.3a).

3 The spring plant biomass was not significantly different among topographical positions
4 (Fig. 3b, Appendix Table A1, $P = 0.590$). The WH system produced the highest plant biomass
5 of the studied cover crop systems at each topography ($P \leq 0.01$).

6 Corn grain yields were the highest in depressions followed by summits and slopes
7 (Appendix, Table A3, $P = 0.005$). We observed no significant differences in corn grain yields
8 among cover crop treatments across topographical positions (Appendix, Table A3, $P > 0.05$).

9 10 **3.2. Soil C and N mineralization and microbial biomass**

11 Across all topographical positions, soil N mineralization followed the pattern
12 WH>WK>Rye>NC (Fig.4a&b). Rate of N mineralization in the WH and WK treatments was
13 significantly higher than in the NC treatment; the difference was most pronounced on slopes
14 (Fig. 4a, $P = 0.04$). The topographical positions did not differ in terms of N mineralization
15 levels. No statistically significant effects of cover crop and topography on soil C mineralization
16 rate were found (Fig.4c&d, $P > 0.05$).

17 Soil microbial biomass C was the highest in depressions when compared to slopes and
18 summits (Fig. 4e). Effects of cover crop systems on soil microbial biomass C was only
19 observed in depressions, where microbial biomass followed a gradient WH>WK>NC>Rye
20 (Fig.4e; $P = 0.09$). The WH system had significantly higher soil microbial biomass than cereal
21 rye ($P < 0.05$).

22 23 **3.3. Correlations between the studied soil properties and plant biomass**

24 In the spring, we observed statistically significant correlations between plant biomass
25 and soil inorganic N and soil C mineralization rates at individual topography and all together
26 (Table 2). Soil NH_4^+ content was positively correlated with total plant biomass ($r = 0.294$; $P =$

0.016 in depressions; $r = 0.432$; $P < 0.0001$ in summits; and $r = 0.206$; $P = 0.003$ for all topographies together, Table 2). Soil NO_3^- content was negatively correlated with total plant biomass ($r = -0.404$; $P = 0.001$ (depressions); $r = -0.336$; $P = 0.004$ (slopes); $r = -0.264$; $P = 0.035$ (summits); and $r = -0.280$; $P < 0.0001$ for all topographies together, Table 2). There were negative correlations between soil C mineralization rates and total plant biomass ($r = -0.456$; $P < 0.0001$ (depressions); $r = -0.402$; $P = 0.001$ (summits); and $r = -0.295$; $P < 0.0001$ for all topographies together, Table 2).

In the fall, soil aggregate sizes of > 2 mm and 0.25-2 mm were significantly correlated with total plant biomass in depressions and all topographies together ($r = -0.315$; $P = 0.026$ and $r = 0.342$; $P = 0.016$ for >2 mm and 0.25-2 mm aggregates, respectively (depressions), Table 2; and $r = -0.155$; $P = 0.061$ and $r = 0.175$; $P = 0.035$ for >2 mm and 0.25-2 mm aggregates, respectively for all topographies together, Table 2).

Soil microbial biomass was significantly negatively correlated with soil N mineralization rates in depressions and summits, and when all topographies combined ($r = -0.282$; $P = 0.022$ (depressions); $r = -0.283$; $P = 0.017$ (summits); $r = -0.151$; $P = 0.028$ for all topographies together, Appendix Table A4). Soil microbial biomass C significantly correlated with soil aggregations in summits ($r = 0.374$; $P = 0.005$ for > 2 mm aggregates; $r = -0.423$; $P = 0.001$ for 0.25-2 mm aggregates; $r = -0.359$; $P = 0.007$ for 0.053-0.25 mm aggregates, Appendix Table A4).

4. Discussion

In the studied edaphic and topographical conditions, in the years with above-average spring precipitation, cover crops did not decrease spring soil moisture for the subsequent soybean crop, regardless of topographical position. While topography generated an expected gradient in soil moisture distribution with depressions being the wettest and slopes the driest,

the cover crop systems did not affect spring soil moisture (Fig. 1a). Live cover crops can decrease soil water content through water uptake and transpiration (Unger and Vigil, 1998; Nielsen et al., 2002); however, differences in soil moisture can be negligible during the growing seasons with average or above-average rainfall (Wortman et al., 2012). The cumulative precipitation during the one-month period prior to the sampling was equal to 128 mm in 2019 and 126 mm in 2020 (Appendix, Fig. A3), typical for wet springs of the US Midwest. There were at least 2-3 precipitation events every week of May 2019, while in 2020 several large rainfall events occurred immediately prior to sampling. Higher aboveground biomass in the WH treatment as compared to the other systems (Fig. 3b) suggests potentially greater water uptake by WH plants, yet abundant spring precipitation likely masked this contribution to soil moisture. Our results agree with the work of Payero et al. (2021) on the effects of single species and cover crop mixtures on soil water in cotton (*Gossypium hirsutum* L.) production systems in South Carolina. Their research indicated that soil moisture was not affected by cover crops under humid conditions (1400 mm of rainfall accumulated during the growing season from Jan 2015 to Jan 2016) (Payero et al., 2021). In addition, our findings are in accordance with previous studies examining soil water in response to cover crop mixtures and individual species (Kuykendall et al., 2015; Nielsen et al., 2015) where no differences in soil water use between cover crop mixtures and single species was reported.

The effects of cover crop systems on soil available N and N mineralization levels did not differ among topographical positions. Higher soil NO_3^- contents in the WK system suggests a promising option for providing more N to the main crop (Fig. 1f). An increase in soil NO_3^- content of WK plots likely resulted from decomposition of Brassica and legume (winter pea) residues, consistent with other studies reporting positive effects of legumes on soil NO_3^- and N mineralization due to their high N content and low C/N ratio (Jahanzad et al., 2016; Finney et al., 2016; Melkonian et al., 2017). Soil N mineralization depends on the chemical composition

of plant residues or organic amendments (Oglesby and Fownes, 1992; Marzi et al., 2020), in which high N-containing organic matter with low C/N ratio stimulates microbial N mineralization and releases more N relative to organic materials with low N content and high C/N ratio (Chen et al., 2014). Higher N mineralization rates observed in WK and, especially, WH systems compared to that of the NC system across all topographical positions also likely result from the legacy residue decomposition in WK mixture and from N inputs from the growing legume (crimson clover) in the WH mixture. Jahanzad et al. (2016) found much faster N mineralization rates due to the presence of the winter pea and forage radish (*Raphanus sativus* L.) cover crops with C/N ratios of 15 and 12, respectively, when compared to cereal rye with C/N ratio of 22. However, a large amount of plant available NO_3^- remaining in the soil under WK systems suggests a possibility of soil N leaching, thereby increasing the risk of environmental pollution. In contrast, the WH and cereal rye cover crops were effective in scavenging soil mineral N, consistent with previous studies reporting high N uptake of ryegrass (Clark, 2007; Chatterjee and Clay, 2016; Behnke et al., 2020). The negative correlation between cover crop biomass and soil NO_3^- indicates uptake of available soil N by cover crop plants for biomass production (Table 2). Additionally, ryegrass and cereal rye can develop dense root systems, thereby potentially preventing nutrient leaching and soil erosion (Kristensen and Thorup-Kristensen, 2004), which is particularly important in agricultural fields with undulating topography and in the wet springs of the US Midwest.

The influence of cover crop systems on soil structure, as expressed through aggregate-size distribution data, was detected only in topographical depressions, where the WH system favoured formation of <2 mm aggregates, while decreasing the fraction of >2 mm aggregates. An increase <2 mm aggregates may improve soil nutrient retention and soil C sequestration as soil organic C might be easier lost from the soils dominated by large compared to small aggregates (Eynard et al., 2005; Devine et al., 2014). Therefore, interseeding the WH cover

1 crop mixture could be an effective strategy in rapid improvement of soil structure, yet the
2 benefits might be present only in some portions of the landscape. The effects of cover crop and
3 topography on soil aggregation were not consistent with spring cover crop growth, since WH
4 had very similar cover crop biomass in all topographical positions, and somewhat numerically
5 lower in depressions. Apparently, it was the intensive WH cover crop growth in depressions in
6 the fall of the previous year that caused the differences in aggregation observed in the spring.
7 Roots influence soil aggregation through physical enmeshment of soil particles as well as
8 exudates and mucilage that act as binding agents combining soil particles, especially in the
9 rhizosphere soil (Bronick and Lal, 2005, Ritz and Young, 2011; Morris et al., 2019). Prolific
10 growth of WH mixtures in summer and fall probably led to more exopolysaccharides produced
11 by enhanced root exudation and/or microbial activity in the rhizosphere, thereby improving
12 soil structure (Lynch and Bragg, 1985; Alami et al., 2000). Biomass of crimson clover was
13 particularly high (about 97 kg ha⁻¹), and clover roots are known for their beneficial effect on
14 soil structure (Mytton et al., 1993, Holtham et al., 2007). The plant growth advantages in
15 topographical depressions disappeared in spring; and in May the WH cover crops in
16 depressions had comparable aboveground biomass to those in slopes and summits, the outcome
17 of likely wetter and colder soil in lower topography (Fig.1a, Luo et al., 2013, Ashiq et al.,
18 2021). However, the roots overwintered and probably the presence of live roots in the soil that
19 was subjected to wetting/drying and freezing/thawing cycles was one of the contributors to soil
20 structure stabilization (Bodner et al., 2013). What made those contributions highly pronounced
21 was finer soil texture in depressions with greater presence of clay and silt (Table 1). Plants
22 grown in finer-textured soil tend to have thinner roots (Helliwell et al., 2017, Phalempin et al.,
23 2021), and root systems with thinner and longer roots contribute to formation of finer soil pores
24 (Bodner et al., 2014), enhancing aggregate formation. Furthermore, soils with higher clay

content and higher soil organic matter generally have a much stronger binding of primary soil particles leading to ubiquitous aggregate formation (Tisdall and Oades, 1982).

Higher soil microbial biomass C in the WH and WK systems was observed only in depressions. It could likewise be an outcome of the presence of Brassicas and legumes in the rotation with their low C/N ratio (as compared to rye), and higher moisture and finer soil texture in depressions – all conditions conducive for microbial growth (Borowik et al., 2016; Kaye et al., 2019; Xia et al., 2020; Muhammad et al., 2021). Previous studies showed that labile soil C and soil microbial functions such as soil N fixation and N mineralization increased in cover crop-based fields (Steenwerth and Belina, 2008; Austin et al., 2017; Balota et al., 2014; Strickland et al., 2019; Perrone et al., 2020; Li et al., 2021). For example, a one-year study of a vineyard field by Steenwerth and Belina (2008) reported that cover crops increased soil microbial biomass C, dissolved organic C, and CO₂ efflux by 1.5-4 fold when compared to treatments without cover crops. A meta-analysis of soil microbial biomass in response to cover cropping by Muhammad et al. (2021) indicated a 24-51% increase in microbial biomass C and N compared to fallow. Higher soil microbial biomass under WH systems in topographic depressions could be attributed to rhizodeposition during prolonged root growth, including root exudation, sloughed cells, and root hair turnover (Austin et al., 2017; Bradford et al., 2013; Strickland et al., 2015; Kallenbach et al., 2016).

We did not observe significant differences in soil C mineralization among cover crop treatments. This contradicts other studies that reported significantly higher C mineralization in treatments with a cereal rye cover crop (Negassa et al. 2015; Finney et al., 2016). However, it should be noted that here we are observing the outcome of only a single season (November-May) of a cereal rye cover, while most other studies reported the results of at least 3-5 years of rye cover crop implementation.

5. Conclusions

Cover crop mixtures interseeded into early standing corn had good establishment and produced sizeable aboveground biomass by the time of corn harvest. The cold tolerant mixture produced substantial biomass in spring compared to relatively minor spring growth of cereal rye cover. Interseeding cover crops into corn at V3-V6 growth stages did not influence corn grain yields. Regardless of topography, interseeded cover crops did not result in topsoil water depletion and competition for water with cash crops in the studied years with above-average spring precipitation. Thus, interseeding can be a promising method of cover cropping in corn-soybean-wheat organic transition rotation system. The WH mixture was the most appropriate option to provide vital benefits to biological, chemical, physical components of soil health in the first year of organic transition. It was effective in soil N capture for subsequent main crops, thereby minimizing soil N leaching potential, and increased soil N mineralization rate. It also increased soil microbial biomass and enhanced soil aggregation. However, magnitude of these benefits was mediated by topography.

Acknowledgments

We would like to thank Maxwell Oerther, Nick Candela, and Nick Barron from the Department of Plant, Soil and Microbial Sciences, Michigan State University for substantial help in data collection, field sampling, and laboratory measurements. We also would like to thank Josh Dykstra and Brook Wilke from Kellogg Biological Station, Michigan State University for help with field data collection and management of the experimental site.

This work is part of the organic transition project funded by USDA-NIFA, Award no. (2018-51106-28779) “Transition to organic in row-crop systems: enhancing sustainability in topographically diverse Midwest landscapes”. Support for this research was also provided by the USDA Long-Term Agroecosystem Research (LTAR) Program and the NSF Long-Term

Ecological Research Program (DEB 1832042) at the Kellogg Biological Station, and by
Michigan State University AgBioResearch.

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