

**Supporting Information.** Jennifer Blesh. 2019. Feedbacks between nitrogen fixation and soil organic matter increase ecosystem functions in diversified agroecosystems. *Ecological Applications*.

## Appendix S1

### Detailed methods for carbon (C) mineralization and *B*-value determination to estimate vetch N<sub>2</sub> fixation.

#### *Short-term C mineralization*

C mineralization (C-min) was determined by a short-term aerobic incubation of rewetted soil (i.e., the flush of CO<sub>2</sub> during a 1-day incubation). In brief, 10 g of air-dried soil was weighed into 50mL centrifuge tubes with lids fitted with airtight, rubber septa. Deionized water was added to each tube to bring the samples to approximately 50% water-filled pore space (WFPS). The CO<sub>2</sub> concentration was measured by sealing tubes, and then immediately removing 0.5mL of headspace gas (time zero) with a syringe and injecting it into a Li-Cor LI-820 infrared gas analyzer (Li-Cor Biosciences, Lincoln, NE). Sealed tubes were then incubated for 24 hours in the dark at 25 °C. At 24 hours, tubes were removed and one-day CO<sub>2</sub>-C was determined as the difference between the time zero and day one CO<sub>2</sub> concentrations.

#### *B-value determination*

The *B* value quantifies the <sup>15</sup>N fractionation that occurs during legume N<sub>2</sub> fixation, which is affected by both rhizobial strains and internal translocation of N from roots to shoots. Vetch seeds were surface sterilized in 70% (v/v) ethanol for three minutes and rinsed three times with deionized water. Seeds were then soaked for an additional three minutes in 3% (v/v) NaOCl and rinsed three more times with deionized water. The seeds were coated with 1g of the recommended N-Dure® inoculant. Four replicates of each variety were planted (16 seeds/pot, thinned to four plants per pot) in a N-free, autoclaved perlite/sand media (1:5 perlite: sand) in pots that had been soaked in 3% NaOCl. Pots were arranged in a randomized complete block design on a greenhouse bench with 16-hr day length, a daytime temperature of 25°C, and nighttime temperature of 15°C. Plants were watered with deionized water, and were fertilized with a N-free nutrient solution. Whole plants were harvested when almost all plants were flowering, separated into roots and shoots, dried, finely ground, and analyzed for δ<sup>15</sup>N.

**Table S1.** Regression coefficients for regression analysis of total fixed N in legume aboveground biomass (in kg N ha<sup>-1</sup>) in 2016, in mixture (vetch-mix) and monoculture (sole vetch), using baseline soil properties as predictors. Coefficients in bold font are significant\*, and the estimated model fit is indicated by the R<sup>2</sup> and adjusted R<sup>2</sup>.

**Model 1**

	Intercept	ln(free POM N) mg kg soil <sup>-1</sup>	ln(protected POM N) mg kg soil <sup>-1</sup>	Bray-1 P mg kg soil <sup>-1</sup>	R <sup>2</sup>	Adjusted R <sup>2</sup>	N	Model P-value
Vetch-mix	170.44	7.08	<b>-44.16**</b>	<b>0.47**</b>	0.47	0.43	40	<0.0001
Sole vetch	159.00	12.63	<b>-41.39*</b>	<b>0.80***</b>	0.42	0.37	40	0.0002

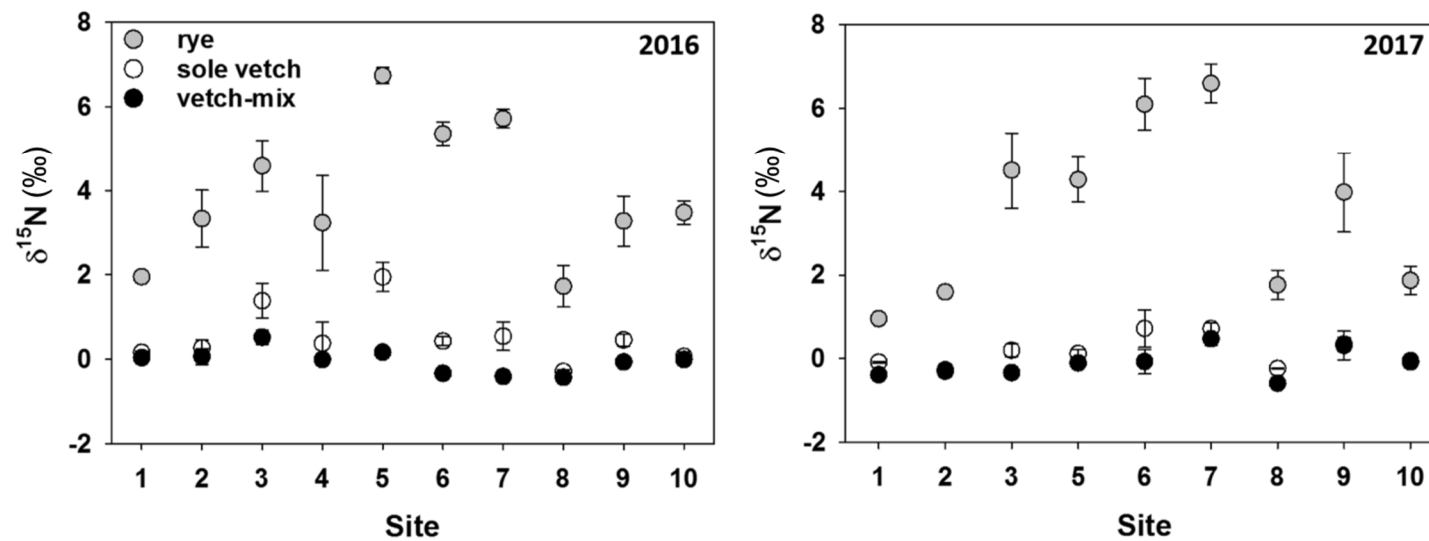
**Model 2**

	Intercept	C:N protected POM	Bray-1 P mg kg soil <sup>-1</sup>	R <sup>2</sup>	Adjusted R <sup>2</sup>	N	Model P-value
Vetch-mix	-126.5	<b>9.32***</b>	<b>0.50**</b>	0.54	0.51	40	<0.0001
Sole vetch	66.7	5.47 <sup>†</sup>	<b>0.87***</b>	0.38	0.35	40	0.0001

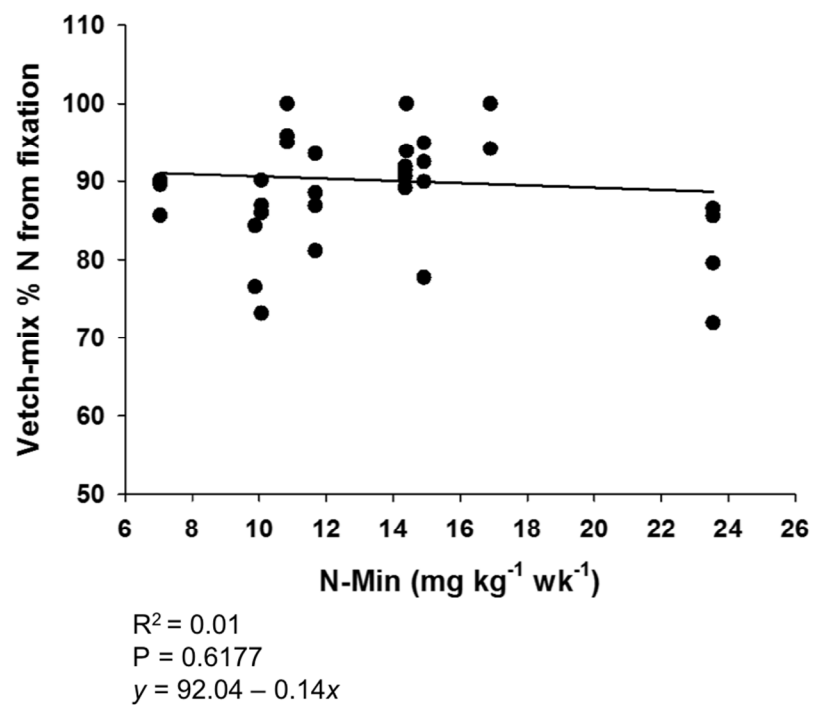
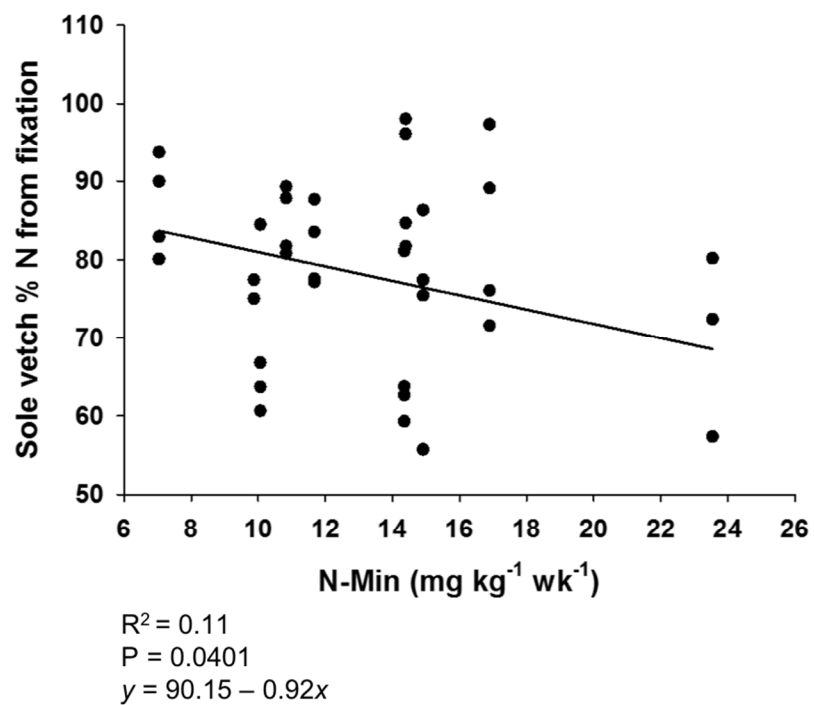
\*Significance: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

<sup>†</sup>Marginally significant (P=0.05)

**Figure S1.** Mean  $\delta^{15}\text{N}$  signature of the reference plant (rye), and of the vetch grown alone (sole vetch) and in mixture with rye (vetch-mix), with standard error, across 10 farms in 2016, and nine farms in 2017.



**Figure S2.** Regression relationships for N mineralization potential and the % of vetch shoot N derived from fixation, for sole vetch (left) and vetch grown in mixture with rye (right).



**Figure S3.** Regression relationships between plant-available soil P concentration and the % of vetch shoot N derived from fixation (top panel), and between soil P and the total amount of fixed N in vetch shoots (bottom panel), for sole vetch (left) and for vetch grown in mixture with rye (right).

