Plant and Soil

Mixed messages: Root traits in cover crops mixtures of blue lupin and winter rye

Roman Kemper*, Thomas F. Döring, Nicole Legner, Catharina Meinen and Miriam Athmann

* Department of Agroecology and Organic Farming, Faculty of Agriculture, University of Bonn, Bonn, Germany, rkemper@uni-bonn.de

Supplementary material

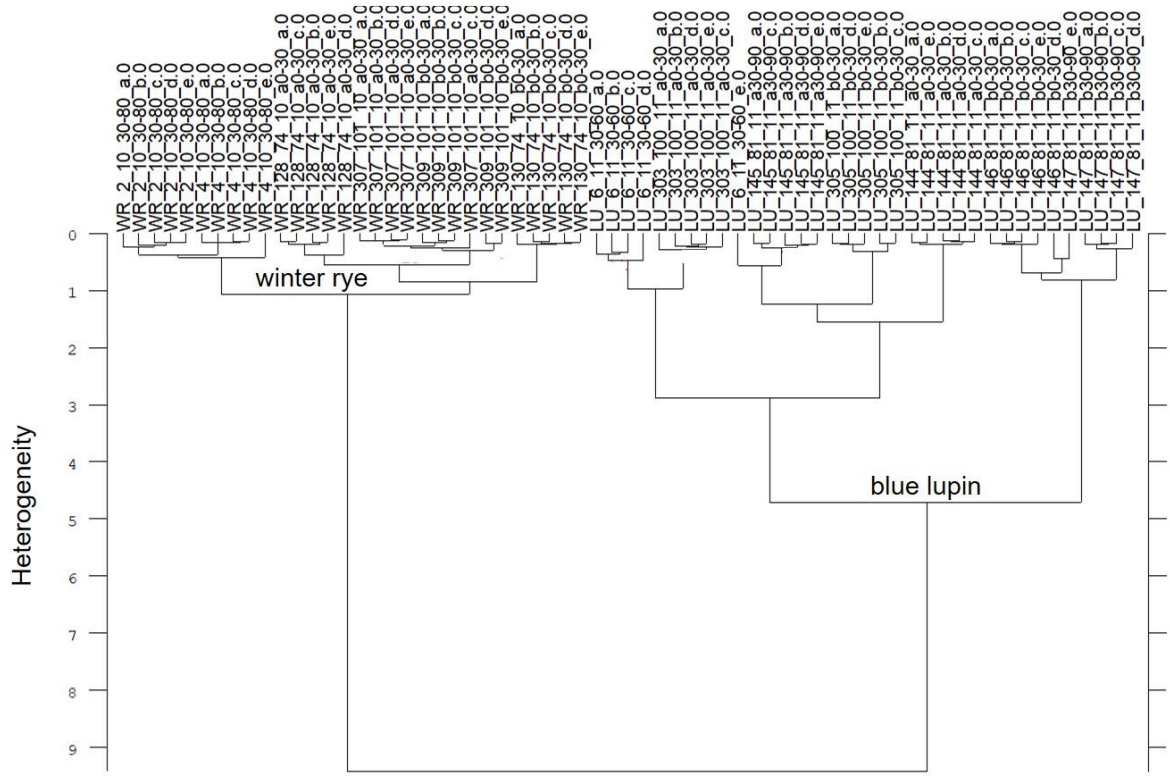

Fig. 1 Cluster analysis of FTIR spectra of single species sample of dried and ground roots of winter rye (WR) and blue lupin (LU) of 2018. File names consist of species name, two (or three) numbers which indicate the plot numbers in the field, the depth of which the samples were taken und replicated measurements (a-e). Cluster analysis was evaluated with the second derivative and vector normalization of the reduced frequency range (1800 – 850 cm^{-1}), Ward's algorithm and Euclidian distance.

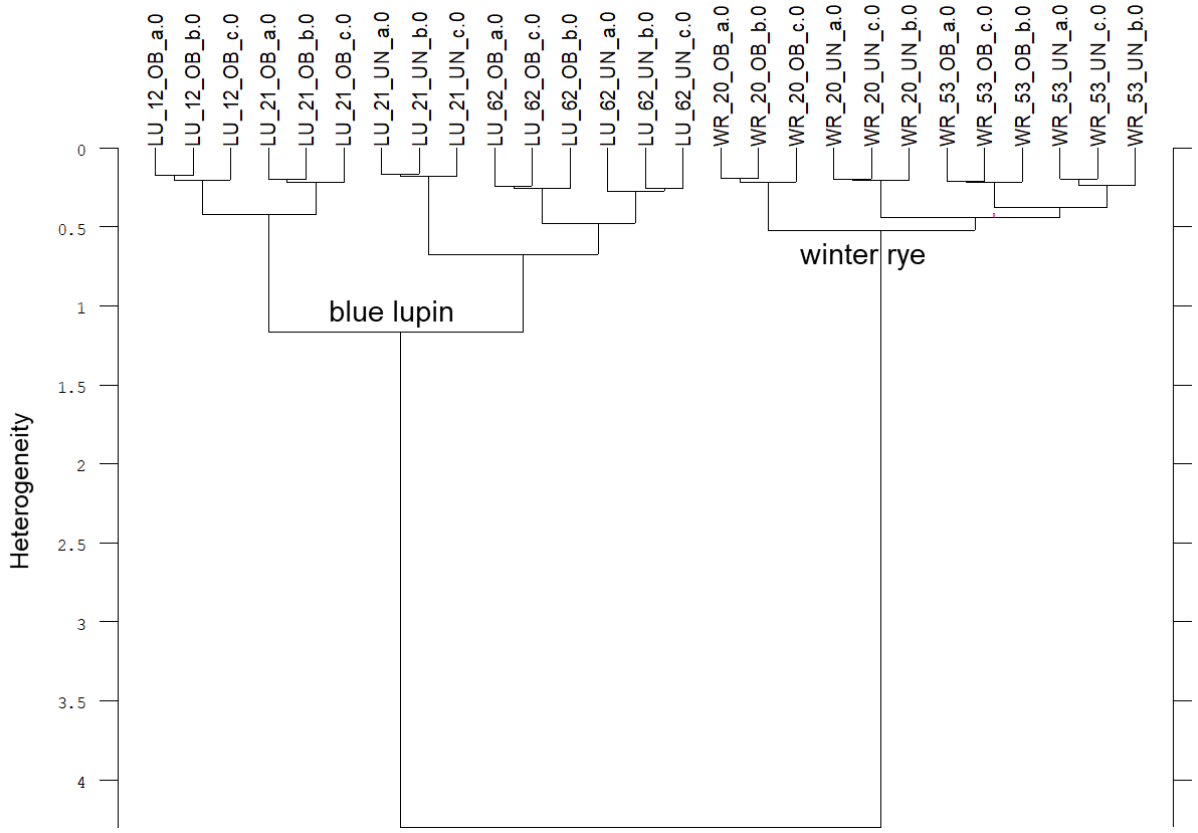

Fig. 2 Cluster analysis of FTIR spectra of single species sample of winter rye (WR) and blue lupin (LU) of 2019. File names consist of species name, the number indicates the plot number in the field, the depth of which the samples were taken (OB means 0-30 cm and UN means 30-90 cm depth) und replicated measurement (a-c). Cluster analysis was evaluated with the second derivative and vector normalization of the reduced frequency range (1800 – 850 cm^{-1}), Ward's algorithm and Euclidian distance.

Details for the model establishing

For establishing a 2-species model, a calibration set of 35 "artificial mixtures" was generated in 3 % steps from 0 % to 100 % for blue lupin and winter rye, respectively. These mixtures covered the complete calibration range thoroughly. 20 additional "artificial mixtures" with known species composition were generated to be used for external calibration of the model. With the FTIR spectra of these calibration mixtures, a model was calculated on the basis of multivariate calibrations with the method of partial least square (PLS) regressions using the software Quant 2 (Opus, version 7.2, Bruker Optics, Ettlingen, Germany). The absorption of infrared radiation is correlated to the concentration of compounds in a multi compound system. The established model was evaluated by an internal validation (cross validation) and was subsequently optimized by the Quant 2 software. This optimization process detects the best data-preparation and the best frequency range to explain the actual mixtures of the calibration samples. Six to eight of the proposed optimized models were verified by an external calibration (for this purpose the 20 additional "artificial mixtures" were used). Both internal validation and external calibration were compared with the calculated statistical parameters of each calibration. For each year a separate model was generated.

The finally chosen model for 2018 as well as for 2019 (Table 1) showed for the calibration/internal validation and external calibration the following different statistical parameters (Table 1): The R^2, the coefficient of determination, was > 97 % in both years. The

RPD, the residual predication deviation, is calculated by the ratio of standard deviation and the standard error of prediction. The RPD should be as high as possible but at least three (Williams and Sobering 1996). Values > 2.5 are satisfactory for screening and values of 5 – 10 are adequate for quality control and > 10 are excellent and equivalent than to reference chemicals (Williams and Sobering 1993). The RPD values of the calibrations are > 6 (Table 1). The RMSECV is the root mean square error of cross validation and accordingly, the RMSEP is the root mean square error of prediction and both values can be to taken to assess the quality of the model. These values should be as low as possible. Due to the optimization process, the models with the lowest RMSECV was chosen. For the chosen models, the values were < 5 (Table 1). The regression line of the calibration illustrates the differences between the true and known values and the predicted values. The intercept and the slope of the regression line can be used to assess the quality of the model. In both years, the intercepts of the calibration were very low with values under 2 (Table 1). The slope for the regression line should be close to 1. Here, regression lines showed values > 0.93 (Table 1, displayed in Fig. 3).
The software is also marking values outside the calibration range. The outside values are values below 0 % or above 100 % which are arithmetically possible but not logical. If one species had 104 %, the corresponding species amounted to -4 %. The values were corrected to 100 % and 0 %.

Table 1 Model details and statistical values for the models of blue lupin (LU) and winter rye (WR) of the years 2018 and 2019. Abbreviation: 1st der. – first derivative, SNV – vector normalization, MSC – multiplicative scattering correction, R^2 - coefficient of determination (in %), RPD – residual prediction deviation, RMSECV – root mean square error of cross validation, offset – offset of the regression line "predicted concentration values vs. true concentration values" for both species (LU and WR), slope– slope of the regression line "predicted concentration values vs. true concentration values" (see also Fig. 3), RMSEP – root mean square error of prediction.

		LU-WR 2018	**LU-WR 2019**
Data pre-processing		1st der. + SNV	1st der. + MSC
Frequency range [cm^{-1}]		4000 – 3280 2560 – 1120	4000 – 3280 1840 – 1120
Calibration/ internal validation	R^2	97.58	97.82
	RPD	6.43	6.77
	RMSECV	4.66	4.42
	Offset LU	1.728	1.344
	Offset WR	1.463	1.478
	Slope	0.968	0.972
External validation	RPD	5.16	5.97
	RMSEP	4.93	4.45
	Offset LU	0.518	2.498
	Offset WR	0.725	4.200
	Slope	0.988	0.933

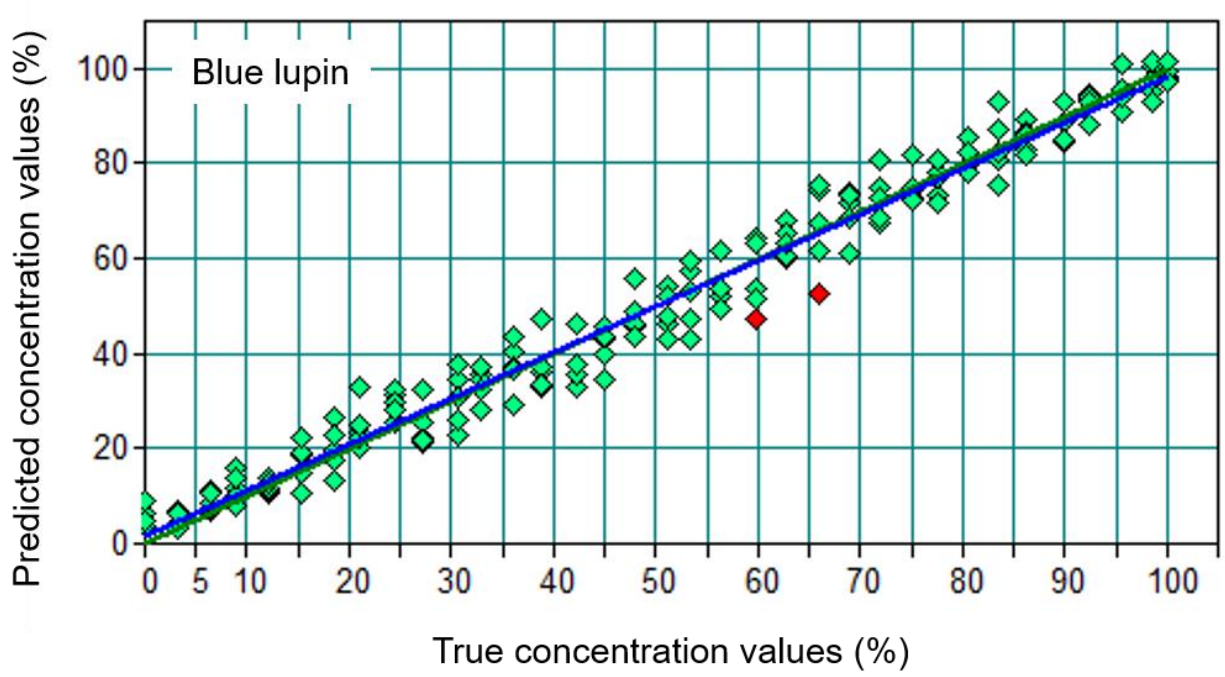

Fig. 3 Predicted concentration values of blue lupin (blue regression line) in comparison to the true (known) concentration values (green dots and green regression line) of the "artificial mixtures" of the model of 2018. The red dots are outlier calculated by the model. The corresponding figure of winter rye of 2018 and for both species for 2019 are very similar and are not shown.

Table 2 Pearson's correlation between relative mixture effect (RME) of root traits and root traits: root length density (RLD), specific root length (SRL) and root mass density (RMD). Significant correlations ($p \leq 0.05$) are given in bold.

RME of root trait	(RME of) root trait	correlation coefficient
RME of RLD	RLD	**0.25**
RME of RLD	SRL	-0.14
RME of RLD	RMD	**0.31**
RME of SRL	RLD	**0.30**
RME of SRL	SRL	**0.58**
RME of SRL	RMD	0.055
RME of RMD	RLD	0.1
RME of RMD	SRL	**-0.44**
RME of RMD	RMD	**0.31**
RME of RLD	RME of SRL	-0.049
RME of RLD	RME of RMD	**0.71**
RME of SRL	RME of RMD	**-0.38**

Williams PC, Sobering DC (1993) Comparison of Commercial near Infrared Transmittance and Reflectance Instruments for Analysis of Whole Grains and Seeds. Journal of Near Infrared Spectroscopy 1:25–32. doi:10.1255/jnirs.3

Williams PC, Sobering DC (1996) How do we do it: a brief summary of the methods we use in developing near infrared calibration, In: Near Infrared Spectroscopy: The Future Waves, NIR Publications, Chicester UK, pp 185–188