Quantification of the ecophysiological advantages of mycorrhiza-maize symbiosis: A promising approach for sustainable food production systems

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Abstract

Purpose

Plant-Soil – "Arbuscular Mycorrhizal Fungi (AMF)" system dynamics are driven by complex arrays of simultaneous cause-effect relationships. Understanding this complexity requires high sophisticated analytical tools and methods such as Structural Equation Modeling (SEM). There has been no practical solution to determine plant-fungus coexistence efficacy. Therefore, the objective of this study is to find a multidisciplinary method to determine the contribution of AMF in coexistence with maize plant.

Methods

Confirmatory factor analysis divided the variables into two groups. On the ecophysiological basis, SEM was employed to above- and belowground relationships in corn-mycorrhizae fields. A SEM model was formulated including the path for cause-effect processes of capture and utilization of resources. The model was satisfactorily calibrated and validated.

Results

Applying multiple regression revealed that variables including leaf area index, stem diameter, dry matter, SPAD readings, plant height, canopy temperature have had the most causal effect to forming corn yield under field condition of inoculation by AMF. *RMSEA* = 0.14 and *normalized chi-square =* 1.97 indicated the model competence. The direct advantages of AMF symbiosis make an increase of 35 percent in resources capture (radiation and from the soil) by association.

Conclusions

These results could be used to manage crop producing systems according to ecological guidelines and environmentally sound operations. We recommend SEM as a crop-soil-AMF system quantifying tool for analyzing treatment effects also for complex arrays of management objectives. The method can employ to determine the efficacy of crop-AMF coexistence which in turn reveal related advantageous may resulted in widespread applying AMF in agroecosystems.

1. Introduction

AMF is an important part of the soil biological system, much of beneficial microbial co-operations throughout the rhizosphere attributed to it (Andrade, 2004; Smith and Read, 2008). AMF symbiosis with plants roots plays a key role in soil productivity and sustainability of terrestrial ecosystems as well as agroecosystems (Mader et al., 2002; Lambers et al., 2008; Van Der Heijden and Horton, 2009; Wagg et al., 2014). AMF form mutualistic relationships with 80–90% of all terrestrial plants, can alleviate prevalent environmental and health concerns related to intensive high input agriculture (Lambers et al., 2008; Parniske, 2008; Gianinazzi et al., 2010; Gaungi et al., 2019). Many species of soil fungi and bacteria are able to solubilize phosphorus *in vitro* and some of them including AMF can mobilize phosphorus in plants. Phosphorus is the second essential element that play a critical role in plant development and growth. The use of AMF plays a key role in improving the growth and the crops yield. It has been reported that the application of AMF improved growth and physiological parameters through rising the activities of antioxidant enzymes and reduction of the oxidative damage caused by the salt stress on *Stevia rebaudiana* (Janah et al., 2021). Vega et al. (2021) reported that the combined use of sustainable tools such as Si fertilization and AMF could be suggested as a promising strategy to overcome the negative effects produced by water deficiency–related stress.

Nowadays, searching for management strategies that are capable of improving phosphorus fertilization efficiency, increase crop yields and reduce environmental pollution caused by phosphorus loss from the soil is of a great interest, due to the problems associated with the restriction of supply phosphorus fertilizer, its rapid fixation in agricultural soils, high costs and environmental pollution caused by it (Alori et al., 2017). The use of mycorrhizal fungi as biofertilizers in agro-ecosystems could be an appropriate solution to this problem. In any coexistence relationship, it is crucial to determine the relative contribution of the two living organisms involved to manage the relationship. In other words, the identification of pros and cons, or costs and benefits of the relationship reveals whether or not the relationship is in natural equilibrium. The overall efficacy of mycorrhizal symbiosis is determined by two components including infectiveness (the rate of infection of the host plant by the fungus) and effectiveness (the rate of the fungus infection could be affecting the host plant performance positively mainly through the ecophysiological mechanisms/processes) (Lambers et al., 2008; Gianinazzi et al., 2010; Jahan, 2012; Schutz et al., 2018; Ganugi et al., 2019). The only conventional method which is being used today just focuses on measuring the level of infection. It has been employed during the past decades as "Determining the percent root length colonization" and is based on the direct observation of fungal organ structures in the root cells of the host plant using binocular. The grid-line intersect method requires fixing and dyeing the host plant root cells, and determining the percentage of host plant root length that contain the fungal structures including mycelia, vesicles and arbuscules (Giovannetti and Mosse, 1980). This method is not only inaccurate, but also is time consuming, laborious and costly. Moreover, possible dying of the root cortex vessels and other related structures simulating false mycorrhizal structures, could be a serious problem. In addition to the above problems, the most important disadvantage of the method, which highlights the necessity of searching for a new and efficient method, is to ignore the determination of the extent of mycorrhizal fungal effectiveness. Allen (2001) showed that root length colonization percentage is not an appropriate index for determining the mycorrhizal infection. Although the efficacy of this method was questioned by some researchers later (Allen, 2001; Hart and Reader, 2002; Jahan and Nassiri-Mahallati, 2013), it is still the only common method, and no alternative has been found. The method proposed in the present paper, unlike the traditional one which is structural oriented (It does not distinguish between natural, neutral and parasitic coexistence relationships) is based on the ecophysiological mechanisms and processes of the host symbiont plant. In other words, it is functional oriented. In addition to fungal structures, this method considers a range of growth and ecophysiological characteristics of the host plant as a functional consequence of its symbiosis which is revealed in plant performance. Unveiling the underlying mechanisms and processes, together with the mathematical relationships between them, helps to quantify the contribution and evaluate the level of usefulness of mycorrhizal symbiosis.

Structural equation modeling (SEM) is sometimes called a “second generation” multivariate method as it provides some advances beyond other so-called “first generation” multivariate methods such as: canonical correspondence analysis (CCA), principal components analysis (PCA), nonmetric multidimensional scaling (NMS), multiple regression (Fornell, 1982; Grace, 2006) .As detailed by Grace (2006), SEM goes beyond the descriptive nature of first generation approaches by introducing confirmatory tests and testing of multivariate hypotheses. It is a highly flexible method applied to test networks of causal relationships. SEM brings researchers closer to causal understanding (as used by Shipley, 2000) by fitting data to models representing hypotheses according to correlative information by determining goodness-of-fit as well as comparing fits among models representing alternative mechanistic hypotheses. SEM could be the best thought of as a quantitative modeling approach rather than a specific statistical technique. A fundamental premise of SEM is that abstracting systems as probabilistic networks provides scientists a practical and effective way to study cause-effect relationships. While when causality is relatively well-known in an ecosystem, SEM has a great strength of ability to partition direct and indirect effects making distinct multiple pathways by which one entity can influence another. Accordingly, the strength of these various pathways can then be estimated and compared (Lamb, 2010) that is why since 2000, SEM is becoming increasingly popular in ecological researches (de Mazancourt et al., 2013). Before that, standard methods (e.g., ANCOVA, multiple regression) did not act satisfactorily where the goal was to study biological mechanisms leading to an outcome (Lamb et al., 2011). There has been little study on ecological aspects of cropping systems using SEM.

The physiological, biochemical, and molecular mechanisms underlying this synergistic action remain poorly understood. The ecological interactions between crop plants and AMF, especially the dynamics of symbiosis are still largely unexplored. Therefore, the main objective of this study is to explore a way to go beyond the solely observation and evaluation of fungal structures in the host plant in order to assess the efficacy of the plant-fungus symbiosis. This multidisciplinary approach consisting of plant ecophysiology, AMF ecophysiology, soil ecology, multivariate statistical methods and mathematical modeling should reveal the underlying ecophysiological mechanisms/processes of the host plant, which are influenced by ecological conditions and entity of the symbiont fungus. It is hoped that this innovative approach enhances our understanding of plant-AMF coexistence in practice and shed lights on more detailed conceptualization of the mechanisms involved, which in turn would be led to more productive -sustainable environment and food production systems.

2. Materials And Methods

2.1. Site description

Field studies conducted at the Research Farm of Agriculture Faculty, Ferdowsi University of Mashhad, Iran (latitude: 36° 15¢ N; longitude: 59° 28¢ E; elevation: 985 m above sea level.). Experiment station was located in Kashaf River watershed in northeast of the country in a semi-arid region (Fig. 1) with mean annual precipitation of 252 mm and temperature of 15° C. Soil samples were taken at 0–15 and 15–30 cm depths and analyzed for some physiochemical properties before conducting the experiment (Table 1).

Table 1
Soil properties of the experimental field (mean of two years).

	Soil depth (cm)	
Soil properties	**0–15**	**15–30**
Total N (%)	0.078	0.065
Available P (ppm)	23	20
Available K (ppm)	462	448
C/N	12.8	12.2
pH (saturation extract)	7.2	7.1
OC[a] (%)	0.53	0.52
EC[b] (dS m^{-1})	1.2	1.2
SP[c] (%)	23.65	23.90
Bulk density (g cm^{-3})	1.43	1.52
Texture grade	Loamy-silt	Loamy-silt

[a] OC: Organic carbon; [b] EC: Soil electrical conductivity;[c] SP: Saturation percentage

2.2. Experiment design

The experiments were conducted based on Randomized Complete Block Design (RCBD) with split plot arrangement and three replications in two successive cropping years at Research Farm of Ferdowsi University of Mashhad to evaluate the effects of AMF on corn under four different cropping systems including: high, medium, low input and an ecologic system (Table 2) that were allocated to the main plots. Seeds inoculated with arbuscular mycorrhizae fungus (Glomus intraradices) and control seeds (non-inoculated) were allocated to the sub plots. The inoculant was consisted of spores plus mycelia with CFU of 106 per g. Cropping systems specification is figured in Table 2.

Table 2
Inputs consumption and agronomical operations in different cropping systems.

Inputs	Cropping systems			
	High input	Medium input	Low input	Ecologic
1- Soil amendments (times)				
Tillage (Mouldboard plow)	2	1	-	-
Disk	3	3	3	1
Leveller	3	3	2	1
2- $N-P_2O_5-K_2O$ (kg ha^{-1})	220:150:100	170:100:50	120:50:0	-
3- Cattle manure (t ha^{-1})	-	-	-	60
4- Chemical control of plant pests and disease (times)	2	1	-	-
5- Chemical control of weeds (times)	3	2	1	hand control

Comparison of four cropping systems was not of objectives at present study. It is widely accepted that AMF growth and development in ecological systems is higher than high inputs and conventional systems. Since SEM works on correlation and variance-covariance matrices, all data concerned to four cropping systems were used in calculations so the final result (AMF collaboration and resulting 35% increase in resources capturing) was obtained from the average of four cropping systems.

2.3. Crop management

Plots of 2.5×3 m with a distance of 1 meter between, to avoid nutrients mixing due to irrigation were arranged. Manure was well mixed with soil using spade one month before sowing. The nitrogen (N), phosphorus (P) and potassium (K) content of manure were determined 2.36%, 0.59% and 2.08%, respectively. The first split of nitrogen and the total amount of phosphorus were applied to each plot of related systems except of ecologic one at sowing. The inoculum used in this experiment was a mixture of propagules of the two species of AMF (*Glomus intraradices*, *Glomus mosseae*) which were obtained from Soil Biology Research Division of Soil and Water Research Institute (SWRI, Ministry of Agriculture, IR) and corn seeds (*Zea mays* L. CV. Single Cross 704) were inoculated with, in accordance with standard instructions then immediately were sown (SWRI, 2013). The sowing dates (May 2) were the same for two years of experiment. Corn seeds were inoculated with fungus (except the control plots) and planted on rows 75 cm apart with 25 cm between sowing hole on rows (The plant density considered 5 plants per square meter). The experiment sites for two years of trial were different but adjacent, which were under fallow during the last year. Plots were immediately irrigated after sowing and later at 7-day intervals.

The corn was selected because it responds well in forming symbiosis with AMF, also is the second most important crop across the world in supplying food security considering cultivation area and production quantity. Obviously, the suggested method in this study is applicable to all plants that could form coexistence with AMF.

2.4. Measurements & Calculations

Each plot was divided into 2 sections, one for seed yield and its components determinig and one for destructive sampling during the crop growth period. Leaf area and dry matter (Drymatter) yield were measured every 2 weeks. – Leaf area was measured by Leaf Area Meter, Li-Cor, LI-1300, USA. Leaf area index (LAI) was calculated by dividing each leaf area value to unit ground surface area.

- Seed yield (Seedyield) was measured form kept-intact 2 m2 of each plot considering marginal effect. The oven dried plants (at 80 oC for 48 hour) were weighed. Dry matter and seed yield were measured then harvest index was determined.
- To determine the P content of plant tissue (PlantP), samples were ground and prepared by dried digestion then amount of P in sap was measured by Morphy and Riley (1962) method.
- At the end of tasseling stage, the plant height (PlantH) and stem diameter (StemD) were measured.
- During the growth period, maximum photosynthesis rate (Amax μ mol.m-2s-1) was measured using LCi, ADC Ltd., UK. The process was repeated five times.
- SPAD readings (SPAD is an index for leaf chlorophyll content) were measured using SPAD 502, Minolta, Japan. This process was performed weekly according to related standard (Beegle and Lingenfelter, 2016).
- Canopy temperatures (CT) were recorded using “Infra-red thermometer KM 842 Standard Model, Kane-May, England” according to Roth and Goyne (2004) manual.

- Root length within specific volume of soil is called Specific Root Length (SRL). At the end of the growing season root sampling was conducted and SRL (within 625 cm3 of soil) was determined according to Tennant (1975) method.

Determination of root length colonization percentage (Colon) required fixing, dying and observing the colonized root length by stereoscope which applied conformed with Kormanik and McGraw (1982) & Giovannetti and Mosse (1980) gridline intersect method, respectively.

- To measure the rate of soil respiration (SoilRes µmol s^{-1}), a separate chamber (SRS 1000 Soil Respiration Hood, ADC BioScientific Ltd. UK) was connected to LCi device to read CO2 amounts.
- The ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence (Fv/Fm) was measured by OS-30 Chlorophyll Fluorometer, ADC BioScientific Ltd., UK. Measurings were obtained 5 times at flowering stage every 7 days on the third fully extended leaf under the tassel.

2.5. Statistical analysis, calculations & model fitting

A normality test was performed to ensure of the data meets the normal distribution. Transformation was also performed for numerical data where needed. To ensure uniformity of treatment variances, the Bartlett's test was used. Since there was no statistical difference between experiment data of two years, thus the mean of each trait values during two years were reported.

The following analyzes were performed step by step on the data collected from field experiments:

I. Analysis of variance (ANOVA)
II. Mean comparisons (based on Duncan 's Multiple Range Test)
III. Factor analysis
IV. Run the SEM model
V. Evaluate the initial model and refined models (evaluation and calibration)
VI. Validation of the model (calculating the goodness of fit criteria, GOF)
VII. Multiple backward stepwise regression

The ANOVA revealed that the effects of maize biotization with the AMF inoculant on most of maize growth traits were significant ($P \leq 0.01$) (data not shown). Mean comparisons revealed that the values of the studied traits of mycorrhizal maize plants were significantly higher than those of non-mycorrhizal plants (the means employed to follow the analysis). To explore possible alternative hypothesis as it provides the multidimensional framework needed to capture the complexity of ecological networks (Xu et al., 2019) and relationships of the maize plant-AMF symbiosis to determine the efficacy of this coexistence, the data matric used for ANOVA was prepared and introduced to Amos to continue the analysis. At the first step, factor analysis was performed which resulted in two distinguished factors. Then the variables with most loads (weight) on each factor were determined. Following analysis, the first and second factor were determined as resource capture latent construct and resource utilization latent construct, respectively (Table 3).

Table 3
Two factor including crop and soil characteristics were identified by factor analysis.

First Factor	Second Factor
- leaf area index (LAI)	- specific root length (SRL)
- SPAD readings (SPAD)	- plant height (PlantH)
- dry matter yield (Drymatter)	- maximum photosynthesis (Amax)
- root length colonization percent (Colon)	- canopy temperature (CT)
- stem diameter (StemD)	- cob numbers (CobN)
	- seed yield (Seedyield)
	- plant tissue phosphorous percent (PlantP)
	- soil respiration (SoilRes)
	- the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence (FvFm)

To check analysis reliability, it must have shown required precision and accuracy of measurement of variables in each factor. Therefore, Cronbach's Alpha Reliability Test was applied (Bellocchi et al., 2009). Cronbach's Alpha is one of the several indices measuring internal compatibility of questions on a questionnaire. It is also applicable to tests and observable variables within an index or latent construct. When a construct or an index has internal compatibility, it means all questions or construct constitutional variables are highly correlated. Some researchers suggested to ensure construct validity, Cronbach's Alpha should be 70% or higher (Cronbach, 1951; Cramer, 1998). In this study, the resource capture construct was rated 0.77, indicating high substantial reliability. The Cronbach's Alpha for the resource utilization construct rated 0.5083 which increased to 0.7422 after eliminating plant phosphorus content (PlantP), soil respiration rate (SoilRes) and the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence (FvFm) variables.

SEM (known also as synonym for LISREL[1]) was then performed to determine the factor with the most significant influence on maize and mycorrhiza performance considering their ecophysiological basis of growth and development (Lambers et al., 2008). It goes beyond regression analysis. Each SEM involves two types of models: First, the measurement model represents the observable variables that measure latent constructs. Second, it is a structural model that illustrates the cause-effect relationships between the constructs. SEM was then employed to determine which factor had the most significant influence on the performance of corn (the studied ecophysiological traits of corn which were employed in the study as measured variables) and mycorrhiza (measured variables including percent root length colonization: Colon; specific root length: SRL) (Jahan and Nassiri-Mahallati, 2013). Considering the role of each crop growth and development trait to yield formation, more analysis was performed to call resource capture (ResCap) and resource utilization (ResUti) as latent constructs. Although theoretical or latent constructs are not directly measurable, but in classic analysis of experimental designs, certain variables such as biomass, leaf area and yield are measured so characterize the main crop traits that represent plant performance (e.g., resource capturing and utilizing). After assigning all measured variables to two factors (two latent constructs) the next step was defining how these factors do interrelates by path analysis in format of SEM (Grace et al., 2014).

Several models were created to test hypotheses and confirm relationships among observed and latent variables to gain additional insight (Evermann and Tate, 2016). Articulately, sensitive analysis, calibration and validation was performed to obtain the most accurate and efficient model (Evermann and Tate, 2016; Xu et al., 2019). In the following, the first and the second factor (were already determined by factor analysis) were named as resource capture latent construct and resource utilization latent construct, respectively.

Regression coefficients, squared multiple correlation coefficients (*R*), variance/covariance matrices, direct and indirect path coefficients were also calculated (Bellocchi et al., 2009; Grace et al., 2014). Covariance indicates intensity and direction of two variables regarded to each other which are called correlation. Model parameter estimations are based on calculations between variances and covariances (Evermann and Tate, 2016). The model was calibrated by tuning off the measured variables in different construct combinations and evaluation of the resulted coefficients and matrices with of the previous model ones. Subsequently, satisfactory results were obtained by validating the model using the second-year data. Finally, *RMSEA* and *normalized Chi-square* (*CMIN*) as the most common evaluation indices were calculated to assess the model accuracy (Evermann and Tate, 2016; Bellocchi et al., 2009). Minitab® Statistical Software Ver. 17, SPSS® Amos Ver. 21 and MS-Excel Ver. 14 were employed to perform analysis and drawing the figures and tables.

2.6. Model evaluation

The model goodness of fit has been calculated and shown in Tables 7 and 8. The root mean square error of approximation (*RMSEA*) is one of the most important indices for evaluating goodness of fit (Evermann and Tate, 2016). In the present study, *RMSEA* of 0.144 indicates the robustness of the model which is reflected in good competence of measured data with theoretical research model. *CMIN* means *minimum chi-square* which was 150 and *CMIN/DF* showing *normalized Chi-square* was 1.97 in research model. Some researchers believe an amount between 1 and 2 and some others believe 1 to 3 is appropriate for *CMIN/DF* (Bellocchi et al., 2009). The saturated model is the model in which all possible parameters have been estimated. In other words, all relations between variables are set up most possibly. The saturated model is always totally competence with *Chi-square* and degree of freedom equal to zero. Unlike saturated model, the independence model indicates only variances related to measured variables. In other words, there is no relation between variables in independence model (also called zero model). Care must be taken to avoid fall in extra theoretical mathematics and statistical aspects of the SEM, due to possible errors might be resulted from over calculations which could be far away from the objective. To meet the mentioned goal, logical and applied interpretation of the results is expected while preventing its misunderstanding. Moreover, depending on conditions, future model refinements may be needed.

2.7. Identification of a regression function to estimate the final plant production

The seed yield of mycorrhizal inoculated maize correlated with many of the variables measured in the experiment. Multiple regression technique was performed to analyze the relationship between yield and traits affecting it more accurately. At first, all the studied variables were included in the regression model and the coefficient of determination (R^2) of this model was calculated by 0.89. Then, a backward elimination stepwise regression technique was performed to eliminate the variables having partial effect on the seed yield as dependent variable (Y).

[1] LISREL (linear structural relations) is a proprietary statistical software package used in structural equation modeling (SEM) for manifest and latent variables. It requires a "fairly high level of statistical sophistication". the LISREL model, methods and software have become synonymous with SEM.

3. Results

3.1. Factor analysis

Confirmatory factor analysis divided the variables into two groups, already called the first factor including five variables and the second factor including nine variables (Fig. 2). Then variables with the most loading (weight) on each of the groups were dedicated to related factor accordingly.

3.2. The Fitted model

The research model consisting of two latent constructs (Resource capture: ResCap and Resource utilization: ResUtil) is proposed has been thoroughly shown in Fig. 3 in which the standardized values of path coefficients and squared multiple correlation coefficients (*R*) for each variable and constructs are represented.

3.3 Regression coefficients of the model constructs

Comprehending more clearly, Table 4 shows the regression coefficients of the model constructs as independent variable, and measured variables as dependent variable with related standard error (*S.E.*), and critical ratio (*C.R.*) of *t* statistic and their probabilities (*P*). Standardized regression coefficients of the model constructs as independent variable to determine the most effective coefficient more easily also shown in Table 1. The regression coefficients of measured variables including dry matter yield (DM: total aboveground biomass), plant stem diameter (StemD), SRL, leaf chlorophyll content (SPAD) and Colon were significant ($P \leq 0.01$).

Table 4
The regression coefficients (Estimates) and standardized regression coefficients of the model constructs as independent variable and measured variables as dependent variable with related standard error (*S.E.*), critical ratio of *t* statistic and their probability (*P*).

Variables		Constructs	Estimate	S.E.	C.R.	P	Standardized regression coefficients
ResUtil	≤—	ResCap	− .039	.028	-1.416	.157	− .558
CT	<—	ResUtil	-68.726	46.126	-1.490	.136	− .726
PlantH	<—	ResUtil	-595.344	393.571	-1.513	.130	− .885
LAI	<—	ResCap	1.000				.889
Amax	<—	ResCap	4.187	1.335	3.136	.002	.456
Drymatter	<—	ResCap	541.928	82.164	6.596	***	.836
FvFm	<—	ResUtil	1.000				.230
StemD	<—	ResCap	.585	.109	5.372	***	.707
Colon	<—	ResCap	6.585	2.817	2.338	.019	.349
CobN	<—	ResUtil	-18.727	13.997	-1.338	.181	− .394
Seedyield	<—	ResUtil	-95.048	63.529	-1.496	.135	− .759
SRL	<—	ResUtil	-1322.171	916.553	-1.443	.149	− .562
SoilRes	<—	ResUtil	.165	.119	1.390	.165	.460
PlantP	<—	ResUtil	1.623	1.113	1.458	.145	.606
SPAD	<—	ResUtil	-4.861	9.856	− .493	.622	.080

ResUtil (Resources utilization), ResCap (Resources capture), Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).

3.4. Variance- Covariance and Correlation Matrices

Variance and covariance of all measured variables has been shown in Table 5. Coefficients of correlation between measured variables indicating standardized values of covariances have been shown in Table 6.

Table 5
Variance (diagonally elements in table) of every measured variable with related covariance (non-diagonally elements in table).

	Amax	SoilRes	StemD	FvFm	PlantP	PlantH	Drymatter	LAI	Seed yield	CobN	Colon	SRL	SPAD	CT
Amax	1.580													
SoilRes	− .001	.000												
StemD	− .045	.000	.059											
FvFm	− .013	.000	− .004	.008										
PlantP	.007	.000	.000	.000	.003									
PlantH	1.086	− .039	1.192	− .291	− .410	195.20								
Drymatter	-1.52	− .059	29.143	-1.79	-2.610	1326.39	36409.63							
LAI	− .041	.000	.049	− .002	− .005	2.83	46.94	.110						
Seedyield	.501	− .007	.221	− .012	− .058	23.37	171.50	.476	6.77					
CobN	.206	.000	.009	− .003	− .024	3.66	5.28	.000	1.16	.973				
Colon	− .577	.001	.261	.014	− .009	17.26	320.17	.614	1.99	-1.28	30.871			
SRL	9.110	− .180	.143	− .924	-1.019	346.89	2345.90	4.063	58.48	1.775	71.766	2385.682		
SPAD	.716	− .004	.337	− .013	.020	6.24	179.11	.330	3.95	.624	1.764	22.370	7.324	
CT	.028	− .006	− .013	− .038	− .058	18.00	20.44	.140	2.84	.880	− .280	27.998	.120	3.867

Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).

Table 6
Coefficients of correlation (*r*) of measured variables of maize crop and soil.

	Amax	SoilRes	StemD	FvFm	PlantP	PlantH	Dry matter	LAI	Seed yield	CobN	Colon	SRL	SPAD	CT
Amax	1.000													
SoilRes	− .109	1.000												
StemD	− .148	.031	1.000											
FvFm	− .115	.199	− .204	1.000										
PlantP	.097	.256	− .024	.073	1.000									
PlantH	.062	− .372	.351	− .231	− .528	1.000								
Drymatter	− .173	− .041	.627	− .104	− .246	.498	1.000							
LAI	− .098	− .109	.604	− .077	− .263	.612	.742	1.000						
Seedyield	.153	− .349	.349	− .051	− .402	.643	.345	.551	1.000					
CobN	.166	− .039	.038	− .036	− .429	.266	.028	− .001	.452	1.000				
Colon	− .226	.035	.193	.028	− .029	.222	.302	.333	.138	− .234	1.000			
SRL	.148	− .494	.012	− .210	− .375	.508	.252	.251	.460	.037	.264	1.000		
SPAD	.210	− .176	.511	− .053	.135	.165	.347	.368	.562	.234	.117	.169	1.000	
CT	.011	− .420	− .028	− .216	− .525	.655	.054	.215	.556	.454	− .026	.292	.023	1.000

Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).

To determine adaptability of theoretical model to empirical model, comparing values a standardized residual error covariance matrix was calculated and results were shown in Table 7.

Table 7

The matrix of standardized residual error covariance of measured variables of the model.

	Amax	**SoilRes**	**StemD**	**FvFm**	**PlantP**	**PlantH**	**Dry matter**	**LAI**	**Seed yield**	**CobN**	**Colon**	**SRL**	**SPAD**	**CT**
Amax	.000													
SoilRes	− .496	.000												
StemD	-1.229	1.434	.000											
FvFm	− .658	.635	− .774	.000										
PlantP	1.001	− .152	1.430	− .449	.000									
PlantH	− .064	.227	.008	− .181	.051	.000								
Drymatter	-1.443	1.161	.211	.022	.243	.535	.000							
LAI	− .943	.798	− .143	.251	.247	1.089	− .006	.000						
Seedyield	.631	.001	.327	.836	.358	− .166	− .056	1.125	.000					
CobN	.920	.960	− .795	.376	-1.267	− .537	-1.051	-1.322	1.004	.000				
Colon	-1.655	.848	− .360	.501	.606	.338	.066	.152	− .066	-2.127	.000			
SRL	.707	-1.559	-1.404	− .545	− .222	.063	− .071	− .183	.210	-1.239	1.056	.000		
SPAD	1.303	− .403	1.232	.039	1.954	− .401	− .220	− .236	2.485	.911	− .283	.178	.000	
CT	− .323	− .557	-2.072	− .329	− .537	.071	-1.846	− .934	.031	1.103	-1.134	− .742	-1.092	.000

Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).

The values of squared multiple correlation (*R*) have been shown in Table 5, relating to variable groups. These values are in fact the coefficient of dependent variable (ResUtil) and the coefficients of measured variables in all following rows. The first-row value is equal to coefficient of determination (R^2) of regression analysis.

Table 8

Squared multiple correlation coefficients (*R*) between resource utilization construct and measured variables in structural model.

Variable	**Estimate**
ResUtil	.311
SPAD	.006
SoilRes	.212
StemD	.500
FvFm	.053
PlantP	.367
PlantH	.784
Drymatter	.700
LAI	.790
Seedyield	.575
CobN	.156
Colon	.122
SRL	.316
Amax	.208
CT	.527

Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).

3.5. Coefficients of the identified causal paths

Standardized direct effects (which are exactly the same as regression coefficients specified in Fig. 3) have been shown in Table 9 (A). The standardized direct effect of LAI by 0.889 means that increasing one unit in *S.D.* of ResCap latent construct results in 0.889 increase of *S.D.* of LAI. The full path coefficients were obtained from summation of direct and indirect coefficients of every variable path. The standardized values of these coefficients help to better compare of paths (Table 9 (A), (B), (C)).

Table 9
Standardized direct effects (A), standardized indirect effect (B) and standardized full effects (C) for resource capture and resource utilization constructs and measured variables in structural model.

(A(			(B)			(C)		
Variables	**ResCap**	**ResUtil**	**Variables**	**ResCap**	**ResUtil**	**Variables**	**ResCap**	**ResUtil**
ResUtil	− .558	.000	ResUtil	.000	.000	ResUtil	− .558	.000
SPAD	.000	− .080	SPAD	.045	.000	SPAD	.045	− .080
SoilRes	.000	.460	SoilRes	− .257	.000	SoilRes	− .257	.460
StemD	.707	.000	StemD	.000	.000	StemD	.707	.000
FvFm	.000	.230	FvFm	− .128	.000	FvFm	− .128	.230
PlantP	.000	.606	PlantP	− .338	.000	PlantP	− .338	.606
PlantH	.000	− .885	PlantH	.494	.000	PlantH	.494	− .885
Drymatter	.836	.000	Drymatter	.000	.000	Drymatter	.836	.000
LAI	.889	.000	LAI	.000	.000	LAI	.889	.000
Seedyield	.000	− .759	Seedyield	.423	.000	Seedyield	.423	− .759
CobN	.000	− .394	CobN	.220	.000	CobN	.220	− .394
Colon	.349	.000	Colon	.000	.000	Colon	.349	.000
SRL	.000	− .562	SRL	.314	.000	SRL	.314	− .562
Amax	.456	.000	Amax	.000	.000	Amax	.456	.000
CT	.000	− .726	CT	.405	.000	CT	.405	− .726

Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).

3.6. Model evaluation

The value of *RMSE* at 90% of probability level (Table 10), and the value of C_{MIN} (*Chi-square*) (Table 11) indicate that the model had satisfactory accuracy.

Table 10
The values of RMSEA for estimated and independence model, respectively. (LO_{90}, HI_{90} respectively represent the low and the high confidence intervals of *RMSEA* lies within at P = 90%,).

Model	RMSEA	LO_{90}	HI_{90}	P
Default model	.144	.110	.178	.000
Independence model	.246	.219	.273	.000

Table 11
The values of C_{MIN} (*Chi-square*) for estimated, saturated and independence model, respectively. (*DF*, *P* represent degree of freedom and probability, respectively).

Model	N_{PAR}	C_{MIN}	DF	P	C_{MIN}/DF
Default model	29	150.021	76	.000	1.974
Saturated model	105	.000	0		
Independence model	14	349.466	91	.000	3.840

3.7. Identified regression function

The results of multiple regression showed that the measured variables including dry matter yield (X_1), leaf area index (X_2), canopy temperature (X_3), leaf chlorophyll content (X_4), specific root length (X_5), and cob number per plant (X_6) were the most independent variables affecting corn seed yield. These identified variables are highly confirmed with the research results and evidences obtained from plant ecophysiological studies (Lambers et al., 2008). In order to better interpret the results, of course, the unit of each measured variable must take into account.

Equation 1.

$Y = -29.86-0.003\ (X_1) + 3.011(X_2) + 0.329\ (X_3) + 0.376\ (X_4) + 0.019\ (X_5) + 0.476\ (X_6) \quad R^2 = 0.89^{**}$

4. Discussion

Although assigning of the variables to the factors was conducted based on factor loadings, it is also noticed there are remarkable empirical supporting evidences of ecophysiological basis among variables (Lambers et al., 2008). In the following, the best fitted model was selected consists of two measuring models including: 1- The resource capture (ResCap) measuring model, 2- The resource utilization (ResUtil) measuring model. These measuring models were related through the structural model (Grace et al., 2010). The model fitting clearly revealed causality path from resource capture to resource utilization is shown by arrow (Fig. 3). The effect of resource utilization construct on specific root length (SRL: total root length in specific soil volume for instance in 25 cm^3) is -0.562 which indicates one unit increase in standard deviation (*S.D.*) of ResCap results in 0.562 unit of decrease in *S.D.* of SRL. This coefficient for percent root length colonization (Colon) were 0.349. Quite considerable amounts in covariances included: DM, Colon (320.17); Seedyield, SRL (58.48); DM, SRL (2345.90); CT, SRL (27.99); Colon, SRL (71.76); SRL, SPAD (22.37). It was suggested that analysis of variance-covariance matrices provides comprehensive interpretation of changes in path coefficients across the scales (Grace ae al., 2010, 2014; Evermann and Tate, 2016). Plant height (PlanH) was highly correlated with SRL (0.50), CT (0.65), and LAI (0.61).

Subtracting sample covariance matrix from implied covariance matrix results in residual covariance matrix. In resulted matrix, lower residual near to zero, closer theoretical model to empirical model. In other words, the standardized residual covariances comply a normal distribution so when the standardized residual error is bigger than 1.96, it indicates statistically significant difference between implied and sample covariances (Evermann J, Tate M, 2016). The lesser amount (< 1) of covariances like in Colon-Drymatter (0.066), Colon-Seedyield (-0.066), Colon-LAI (0.152), Colon-PlantH (0.338), Colon-stemD (-0.360), Colon-FvFm (0.501), Colon-PlantP (0.606), Colon-SoilRes (0.848) (Table 4), and indicates high correspondence of implied (theoretical) and sample (empirical) covariance matrices. The residual covarinces of SRL-PlantH (0.063), SRL-Drymatter (-0.071), SRL-LAI (-0.183), and SRL- Seedyield (0.210) were also less. These values indicate that colonization of maize root as a result of mycorrhizal inoculation had positive effects on growth traits and overall performance of maize.

The value of *R* = 0.31 of resource utilization as dependent variable indicates that the suggested model explains 31.1% of variations of the resource utility variable. *R* in fact indicates the concept of reliability (Bellocchi et al., 2009). In other words, the ResUtil value is the same as the standardized squared factor loading. For instance, the value of 0.575 for seed yield (Seedyield) means: ResUtil explain 57.5% of seed yield variations. From another aspect, the values of *R* indicate the adequacy of every variable. As shown in Table 8, the major values are more than 0.500 indicating they are ideal indices to assay their own latent construct. Conclusively, variables empowering ResUtil as the final determining factor make higher yield formation. In this study, inoculation of maize plant with AMF makes the dominant ability of ResUtil more effective resulting in higher corn yield. When the correlation coefficient is between 30–50% it means that the observed variable is relatively week, but it could be enough to continue analysis. The values more than 50% mean that the observed variable is eligible to calculate the latent variable (Bellocchi et al., 2009; Evermann and Tate, 2016). It is worth noting, since variables like leaf area index (LAI), plant height (PlantH) mainly defines plant radiation capture ability (Lambers et al., 2008), AMF inoculation indirectly increase also resource capture (ResCap) through corn shoots. Root system development and nutrients capture are physiologically followed by the shoot ability to capture and use radiation (Lambers et al., 2008). Generally, Table 8 indicates high *R* of Seedyield, CT, PlantH with ResUtil construct. Reversely the lower *R* of maximum photosynthesis rate (Amax), Colon and SRL suggest that the final determining factor of corn yield is in fact its ability to utilize resources. As formerly explained, the higher *R* of LAI, PlantH as radiation capture ability had the most impact to determine corn yield (LAI, PlantH potentially define optimum space distribution of leaf area (widely known as "Canopy Architecture" in crop ecophysiology) (Lambers et al., 2008). The correlation coefficients of LAI-Colon (0.33), LAI-SRL (0.25), plantH-SRL (0.50) and plantH-LAI (0.61) which means the effect of AMF is indirect and mainly was realized by increasing LAI and PlantH (Table 6). Many researchers previously proved the AMF symbiosis increases the host plant leaf area, change root/shoot ratio which in turn enhances water uptake, and consequently improving plant tolerance against stresses finally resulted in improved the dry matter accumulation (Lambers et al., 2008; Smith and Read, 2010; Begum et al., 2019).

The most direct effect on ResCap and ResUtil constructs, were related to LAI and plantH (Table 6A). Considering relations between stemD and vascular vessels along physiological relations of sink and source (Lambers et al., 2008(, the importance of stemD in resources capturing reveals more clearly. PlantH plays an important role to more effective radiation capture by leaves. In the other hand, better PlantH normally results better spatial distribution of leaves (Lambers et al., 2008). The highest amount for the ResCap construct after LAI and DM was ranked to StemD (0.707) indicate plant ability for translocation of assimilates (photosynthesis products). The values of 0.349, 0.314 for Colon and SRL indicates that result of inoculation with AMF was slightly more affective in Colon than SRL in resource capture (Table 9A). This collaboration and resulting 35 percent increase in resource capturing by corn seems reasonable. This fact is strongly supported by the other novel findings of our study (Fig. 3).

Coefficients of Eq. 1 show the relative impact of changes in each of the variables in the model on seed yield. Due to the effect of mycorrhizal inoculation, it was possible to quantitatively evaluate the response of maize seed yield on the basis of increasing or decreasing mycorrhizal affected variables. SEM revealed that the consisting variables of LAI, StemD, DM, SPAD, PlantH, CT had the most causal effect on forming corn yield under field inoculation of AMF which is mostly in agreement with the regression results. There are many reports that emphasize the role of mycorrhizal symbiosis in increasing specific root length, increasing water uptake and subsequently decreasing canopy temperature, increasing leaf area and leaf chlorophyll content (as the center of photosynthetic light reactions) (Lambers et al., 2008(, which ultimately leads to increased plant production (Smith and Read, 2008; Lambers et al., 2008; Begum et al., 2019). These effects are well reflected by Eq. 1, which could be confirmed the validity of the present study. As an applied agroecological management tool, Eq. 1 can be employed to corn plant under similar conditions of the present experiment to evaluate the effect of mycorrhizal inoculation on the final product, also to estimate or to predict the seed yield. A critical improvement of the equation would be best achieved by estimating or quantifying the regression coefficient in accordance of local conditions. Applying such an approach is possible for any other plant species after collecting experimental data and performing multiple regression on the data.

Conclusion

This study revealed two mechanism/process-based latent construct of resource capture and utilization and confirmed the quantified cause and effect relations between. Direct facilities resulted from AMF symbiosis was revealed in increase of 35% in resource capturing through collaboration. The fitted model indicated high accuracy and competence (*RMSEA*=0.14). The backward stepwise regression technique truly identified and confirmed the variables whose effectiveness of mycorrhizal inoculation was recognized by SEM. The coefficient of determination of the regression model (R^2=0.89) indicating that this model could explain 89% of the total variance in maize seed yield as the dependent variable. Overall, the function-oriented method employed in this study can be used as an efficient alternative to the conventional method in determining mycorrhizal (also, any co-existence relationship) efficacy. Employing this method for wide range of crop plants, assists the experts and researchers interested in mycorrhizal technology to quantify mycorrhizal effects, which in turn can help farmers, advisory services, researchers and policy makers to provide the necessary practical ground for the widespread implementation of mycorrhizal technology in agroecosystems, to improve food production and productivity based on health, cost and energy considerations as the most challenging issue of our time, that is more important than ever because of the more efficient and sustainable use of resources and the preservation of the environment could be resulted from.

Declarations

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Authors’ contributions

The authors of this research paper have directly participated in the planning, execution, or analysis of this study. The authors read and approved the final edition of the manuscript. CRediT author statement: M.J.: project administration, methodology, investigation, software, investigation, data curation, formal analysis, writing-original draft preparation writing-reviewing and editing. M.N.: conceptualization, validation.

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Availability of data and materials

Additional data may be available on request to the authors; please contact the corresponding author. We are legally responsible for information, data, used methods, and results.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no competing interests. The authors declare that they have no conflict of interest and agree with submission of the manuscript to the journal of Soil Science and Plant Nutrition. All the research meets the ethical guidelines, including adherence to the legal requirements of our country.

Code availability

All used and created codes are available on demand.

Ethics approval

This research meets all the ethical guidelines, including adherence to the legal requirements of my country.

Consent to participate

The authors voluntarily agree to participate in this research study.

Consent for publication

The authors confirm no conflict of interest and agree with the submission of the manuscript to your Journal.

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Figures

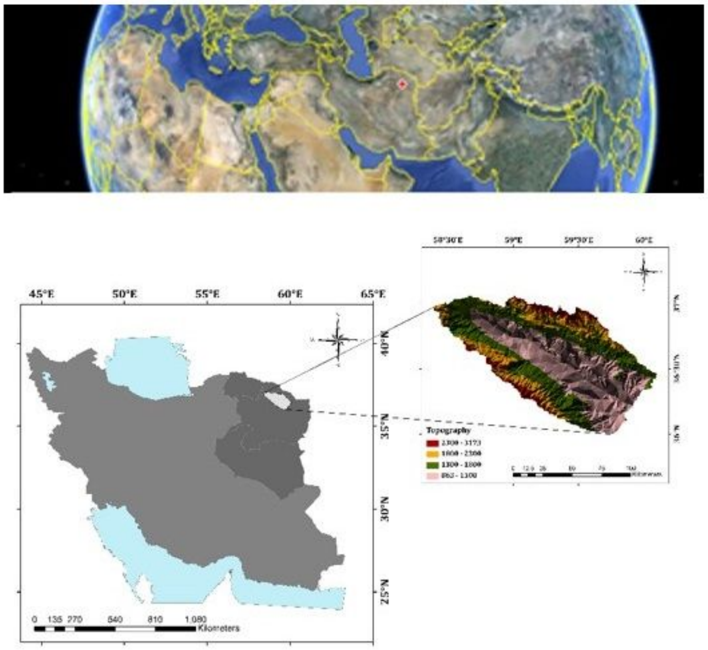

Figure 1

Geographical location of study (Kashaf River watershed at Northeastern Iran).

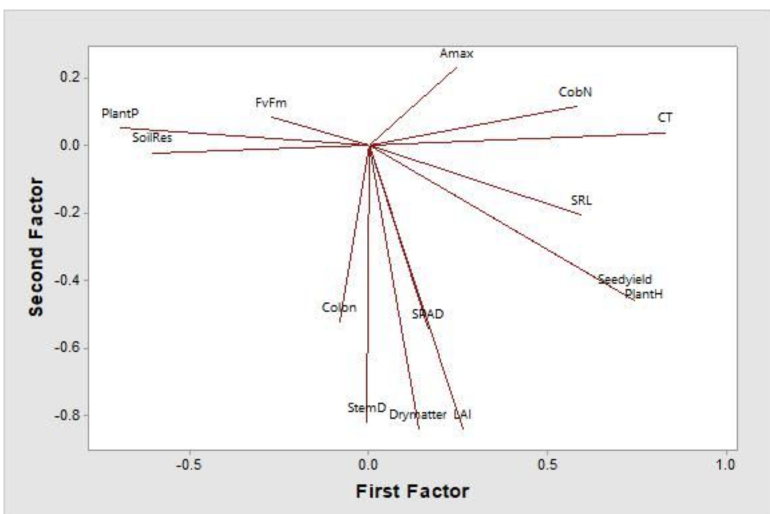

Figure 2

Loading plot of measured variables on the first and the second factor.

Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).

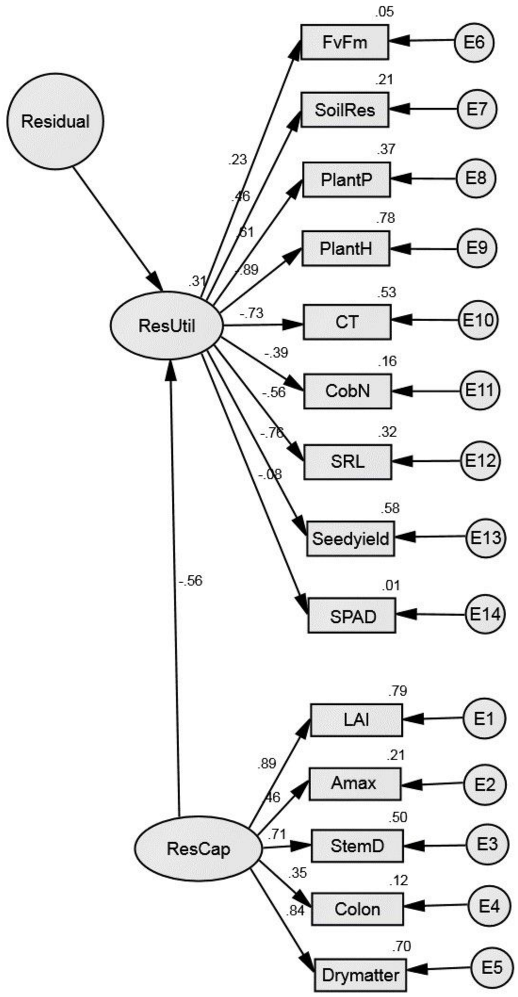

Figure 3

A schematic view of suggested model with estimated standardized parameters (path coefficients have been shown on arrows; coefficients of multiple squared correlation (*R*) have been shown on top right corner of each rectangular).

ResUtil (Resources utilization), ResCap (Resources capture), Amax (maximum photosynthesis rate), SoilRes (soil respiration rate), StemD (stem diameter), FvFm (the ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence), PlantP leaf (plant tissue phosphorous percent), PlantH (plant height), Drymatter (dry matter yield), LAI (Leaf area index), Seedyield (seed yield), CobN (cob numbers), Colon (root length colonization percent), SRL (specific root length), SPAD (SPAD readings), CT (canopy temperature).