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Impact of Long-Term Manure and Mineral Fertilization on Accumulation of Non-Structural Carbohydrates in Lucerne Forage

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Abstract: Fertilization management affects both productivity and nutritive value of forage legumes. However, there are few studies about changes in lucerne non-structural carbohydrates under long-term fertilization. The aims of this study were to compare the effects of mineral fertilization and organic manure on lucerne plant parts (leaf, stem) starch and water-soluble carbohydrate (WSC) accumulation in association with canopy structure following 60 years of different fertilization management approaches. Treatments investigated were: two contrasting levels of mineral N, P_2O_5 and K_2O application (0:0:0 and 91:71:175), each with and without farmyard manure. Changes were mainly reflected in WSC content where intensive mineral fertilization consistently reduced the stem and forage WSC in contrast to unfertilized control or manure alone. These changes could be associated with a dilution effect presented by the highest increase of maximal stem length at these treatments. Manure improved leaf and forage WSC despite the associated increase in maximal stem length and leaf weight ratio, probably as a result of improved soil environment together with the potentially increased presence of arbuscular mycorrhizal fungi. Results showed that manure fertilization has potential for improvement of lucerne WSC, despite some negative relationships between lucerne canopy traits and sugar content.

Keywords: alfalfa; manure; slurry; phosphorus; potassium

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1. Introduction

Fertilization strategy is important for forage growers in terms of the opportunities it provides to influence both biomass yield and quality and thereby ensure profitability of livestock production [1] and the sustainability of forage systems [2]. Studies on the effects and responses to fertilization have mainly focused on the effects of direct application of key macronutrients such as nitrogen (N), phosphorus (P) and/or potassium (K) in different types of fertilizers and various combinations in relation to forage legumes, grasses or legume-grass mixtures performance [3–5]. Considerations of the influence of fertilization on forage nutritive traits have mainly focused on changes in crude protein, fractions of fiber, organic nutrient digestibility and forage intake [6,7]. Although there is a considerable amount of published research on the effects of fertilizers on grassland, including both short-term and long-term investigations, studies with forage crops under field conditions have mainly involved relatively short-term experiments and there is a lack of studies under long-term fertilization management [8].

In the case of lucerne, research on the effects of fertilizer applications has usually found positive yield responses to optimized supply of P and/or K in association with a favorable soil nutrient status [9,10]. Although the N requirements of lucerne plants can be met by symbiotic N_2 fixation, under some environmental conditions additional mineral N fertilization could also be effective in increasing biomass accumulation [11]. There is an inverse relationship between forage biomass accumulation and decline in nutritive value [12]. Lower forage yield under P and K deficiencies also resulted in decreased concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) with an associated positive effect on in vitro digestibility [1]. In line with the effect of added nutrients, Clark et al. [6] observed a tendency toward lower digestibility after slurry application. In addition to direct nutrient application, the indirect effects of nutrients applied over the whole crop rotation period must also be considered. This is important because manure fertilization has potential beneficial effects on lucerne forage quality, despite the possible negative relationships between increased herbage accumulation and forage quality [8].

Among nutritive traits, non-structural carbohydrates (NSC), represented mainly by water-soluble carbohydrates (WSC) and starch, require special attention for several reasons. NSC contents are linked to diurnal variations and higher contents can result in increased forage digestibility; this may be demonstrated by lower fiber fractions and higher forage intake, as well as improved milk performance for forage harvested in the afternoon [13–16]. Enhanced starch content, unlike WSC, can additionally contribute to the synergistic action in the animal's small intestine required for maximal production [17]. Another benefit of higher NSC could be related to their impact on silage fermentation where they represent the key sources of fermentable substrates during ensiling [18], as starch can contribute to lactic acid fermentation after its hydrolysis [19]. Lucerne usually provides lower contents of WSC compared with grasses or red clover, and therefore selection of suitable partner species in the mixture, together with optimization of cutting according to time of day, can be used to increase NSC in the forage [15]. The importance of NSC could also be highlighted in relation to their potential for improvement of protein utilization by ruminants [20], where higher soluble sugars + starch/soluble protein ratio represent the desired synchrony of energy and N compounds in rumen associated with potentially greater N-use efficiency [21]. According to Berthiaume et al. [22], NSC in lucerne is an important determinant of ruminal-N losses when it promotes glucogenic fermentation and enhances microbial N synthesis in the rumen.

Due to these above-mentioned benefits of NSC, there is a logical effort to optimize the management of their content in forage legumes. NSC accumulation varies not only among species, but also among genotypes within species and this had led to efforts to improve their content by breeding, with the highest attention paid to starch in forage legumes [17,23]. Variation among seasons, developmental stages, plant parts and harvest management could all be important for forage fractionation [24] or optimization of harvest timing according to time of day reflecting the natural diurnal fluctuation [15,16], although the benefit of the latter may be reduced by post-harvest losses [17]. Fertilization could also have potential to influence NSC content where increased content of N compounds due to N fertilization is compensated for by reduction in NSC and increased lignification [20]. Reduced leaf and stem NSC by N and P fertilization could also be explained by the dilution effects of increased biomass following fertilization [25–27]. Recent research in lucerne has demonstrated that long-term manure application, in contrast to mineral fertilization, could have some potential for improvement of nutritive value of both leaves and stems, with resulting quality improvement on a whole-plant basis [8]. However, the effects of fertilization on lucerne NSC accumulation under field conditions have received little attention although it could be beneficial due to the importance of the NSC fraction. Therefore, in this study, the aims were to determine the effect of manure and mineral N fertilizer when applied over the whole crop rotation, together with P and K applications in each autumn on: (i) water-soluble sugars and starch in lucerne plant parts and total forage; and (ii) canopy structure traits and per-hectare NSC production, on land that had received different fertilizers over the preceding 60 years. As an additional goal, we sought to evaluate the contribution of changes in canopy traits, caused by fertilization, to the total variability in NSC content. Results from this investigation have potential value in terms of understanding how long-term fertilization management affects NSC accumulation in lucerne.

2. Materials and Methods

2.1. Site Description

The Ruzyně Fertilizer Experiment was established on a permanent arable field in 1955, at the western edge of Prague, Czech Republic (50°05′15″ N; 14°17′28″ E). Site altitude is 338 m, annual mean temperature is 8.5 °C and precipitation sum is 496 mm (average values of 1981–2010, Prague-Ruzyně weather station). The soil is classified as an illimerized Luvisol. The upper 30 cm (arable layer) contains 27% clay, increasing to 40% in the subsoil (soil layer 30–40 cm) and 49% at 40–50 cm depth. The soil pH (H_2O) was 6.5 in the top 20 cm before establishment of the experiment in 1955.

2.2. Experimental Design

The Ruzyně Fertilizer Experiment is a field experiment with five various field strips established in 1955, where 24 fertilizer treatments are replicated four times in a complete randomized block design (with 96 individual plots) in each strip. Each plot is 12 m × 12 m and assessments of crop yield are made from the central 3 × 12 m of each plot. In the present study on lucerne, the following four fertilization treatments were considered: control without any fertilizers (NIL); farmyard manure (FYM) alone; and intensive mineral fertilization (N4P2K2), each with and without FYM. Table 1 summarizes these four contrasting treatments (16 individual plots) with different levels of nutrient supply. Manure was regularly applied in autumn prior to the subsequent planting of beet or potatoes in the crop rotations. Phosphorus (as superphosphate, 19% P_2O_5) and potassium (as potassium chloride, 60% K_2O) were applied annually in the autumn. Mineral N fertilizer was applied at different rates to all crops in the rotation except lucerne. The applied rates of nutrients together with soil analyses in treatments selected for this study are shown in Table 1. The study analyzing lucerne NSC in response to variable nutrient supply was carried out in Strip IV within a 9-year crop rotation system in which one crop is always growing on the whole strip (96 plots). The lucerne (variety Morava, seed rate 15 kg ha^{-1}) was established in April 2012 and subsequently harvested in 2013 and 2014. Additional information on soil, crop rotation, fertilization management, stand establishment and forage harvesting schedules is given in Hakl et al. [8].

Table 1. Average annual rates of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) for mineral and farmyard manure (FYM) fertilization treatments, mean values of soil pH H_2O, K, P, Mg and Ca nutrient status (in mg kg^{-1}) together with soil organic carbon (Cox, %) and total soil nitrogen (Nt, %) assessed in 2014. Plant available nutrient concentrations were determined by Mehlich III extraction procedure.

Treatment	Organic N:P:K (kg ha^{-1})	Mineral N:P:K (kg ha^{-1})	pH	K	P	Mg	Ca	Cox	Nt
NIL	0:0:0	0:0:0	6.2	147	14	149	2957	1.26	0.125
N4P2K2	0:0:0	91:71:175	6.0	174	76	129	2738	1.44	0.137
FYM	30:18:24	0:0:0	6.2	177	37	166	3190	1.51	0.158
FYM + N4P2K2	30:18:24	91:71:175	6.0	258	106	164	2949	1.59	0.149

2.3. Forage Sampling and Measurement

In 2014, forage sampling from the described four treatments took place on 9 May, at the mid-bud stage before the first cut. Biomass from a 0.5 meter-length of a row was clipped in four replicates using hand scissors, to a stubble height of 50 mm. To ensure higher NSC content in the harvested samples, the sampling was carried out after 3 pm (this sampling was independent of the forenoon sampling reported by Hakl et al. [8]). All cutting dates in 2014, together with average cut yields and January to July weekly weather data (mean temperatures and precipitation) are presented in Figure 1. The plant density (plants per m^2) was determined in each sampled area and the number of all stems (stem density per m^2) together with length of the longest stem (maximal stem length, in cm) was determined in the clipped fresh samples. Fifteen representative stems were selected from each sample and leaves (blade, petiole, stipule) were separated by hand from the stems (stem, bud) for assessment of leaf weight ratio (g kg^{-1} DM) after oven drying at 60°C. Dry matter yield (DMY, t ha^{-1}) was calculated from plot fresh matter yield and dry matter content of the particular treatment.

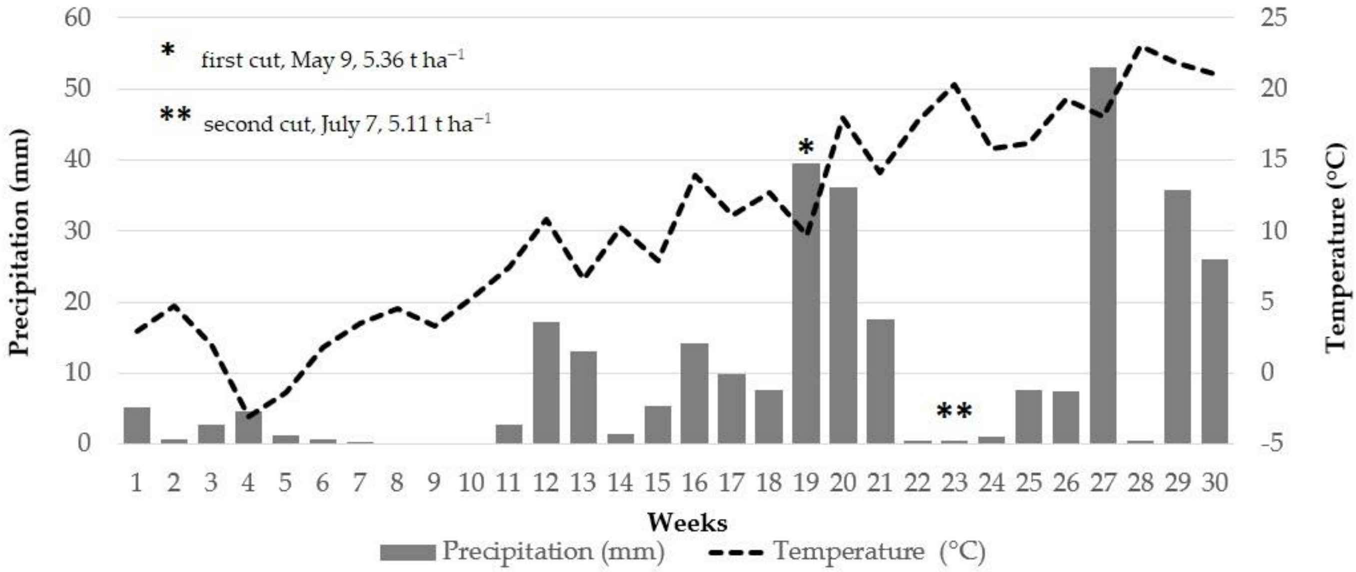

Figure 1. The weekly mean temperature (T, °C) and sum of precipitation (P, mm) for January–July in 2014 together with lucerne harvest date and average dry matter yield (DMY, t ha^{-1}).

Dry forage leaf and stem samples were homogenized to a particle size of 1 mm and analyzed for starch and WSC in the accredited laboratory of Ekolab Žamberk Ltd. (Žamberk, Czech Republic). Starch was measured by polarimetric (Ewers) method, where starch is released from the sample by boiling in dilute hydrochloric acid. Sugar contents were measured by titration according to the Luff–Schoorl method after extraction with 40% ethyl alcohol. The results for the whole harvested lucerne forage were calculated from the nutritive value of leaves and stems in association with leaf-weight proportion. The WSC and starch hectare production were calculated from lucerne dry matter yield, their content in plant parts and leaf weight ratio in each plot.

2.4. Statistical Analysis

Effect of fertilization treatment on WSC, starch, canopy traits and dry matter yield was analyzed by one-way ANOVA with fixed effect followed by post hoc Tukey HSD at $\alpha = 0.05$. All analyses were performed using the Statistica 12.0 [28]. Redundancy analysis (RDA) as implemented in CANOCO 5 [29] was used to perform analyses and to visualize relationships between fertilization treatments (categorical variable), NSC contents (dependent variable) and canopy traits (supplementary or covariate). Analysis A1 investigated the proportion of lucerne NSC variability given by fertilization, while variation partitioning was performed in analysis A2, excluding canopy traits as covariates. Canopy traits were presented by variables in Section 2.3 with an exception for plot DMY, which was replaced for multivariate analyses by leaf, stem and forage yield in each sample (in g m^{-2}). The fertilization treatment and maximal stem length that best captured the variability in our data were tested and selected using the forward selection function, and a Bonferroni correction was applied to reduce the probability of first-order error [30]. The statistical significance of all the constrained canonical axes was determined by the Monte Carlo permutation test (unrestricted 499 permutations). The data of dependent variable were log-transformed y′ = log10 (y + 1) and the option of center and standardization was used in RDA.

3. Results

Lucerne stands survived without any evidence of substantial leaf disease or pest damage. The year 2014 was hot and dry in April but this was compensated for by colder and humid weather in early May (see weekly values in Figure 1). Lucerne leaves were significantly lower in WSC and higher in starch compared with the equivalent values for stems (data not shown).

3.1. Impact of Fertilization on Lucerne WSC and Starch

Effects of included fertilization treatments on lucerne leaf, stem and whole forage WSC and starch contents are summarized in Table 2. Leaf WSC was lowest in the FYM+N4P2K2 treatment, compared with mineral fertilization only or FYM only. Stem WSC was reduced in both treatments with mineral fertilization. None of the four fertilization treatments significantly affected the content of starch in lucerne plant parts. The changes in WSC of leaves and stem resulted in differences in the overall WSC content of harvested lucerne forage in relation to leaf weight proportion. Similar to effects on stem fraction, mineral fertilization resulted in reduced whole forage WSC, with the lowest value observed for the FYM+N4P2K2 treatment.

Table 2. Effect of fertilization treatments on water-soluble carbohydrates (WSC) and starch content in lucerne leaves, stems and whole forage in the first cut 2014.

Treatment	Leaves		Stems		Forage	
	WSC (g kg^{-1})	Starch (g kg^{-1})	WSC (g kg^{-1})	Starch (g kg^{-1})	WSC (g kg^{-1})	Starch (g kg^{-1})
NIL	47.5 ^{ab}	61.4	128 ^{b}	6.00	89.6 ^{c}	32.5
N4P2K2	51.4 ^{b}	59.5	101 ^{a}	5.53	80.8 ^{b}	27.4
FYM	55.2 ^{b}	58.1	126 ^{b}	3.97	96.1 ^{c}	26.9
FYM + N4P2K2	36.4 ^{a}	49.0	92.3 ^{a}	4.60	70.2 ^{a}	22.0
p	0.002	0.600	<0.001	0.715	<0.001	0.193

n = 4; one-way ANOVA; different letters document statistical differences for Tukey HSD, α = 0.05.

3.2. Effect of Fertilization on Canopy Structure, Forage Yield and Carbohydrate Production

Effects of fertilization on canopy traits and forage yield are shown in Table 3. All canopy structure traits were influenced by fertilization; however, the achieved forage yield did not differ among treatments. The lowest plant density was observed in the treatment FYM+N4P2K2, whereas the highest stem density was in the N4P2K2 treatment. Both mineral and manure fertilization were associated with higher stem length together with reduced leaf weight ratio relative to that of the unfertilized control. The highest stem length and lowest leaf weight ratio were both observed in the FYM+N4P2K2 treatment.

Neither the yield in the first cut nor the annual forage yields were significantly different between treatments. Leaf WSC and starch yield were significantly reduced in the FYM+N4P2K2 treatment (Table 4). Stem WSC yield was lower after mineral fertilization in comparison with manure alone. Stem starch yield was reduced for FYM compared with the NIL and N4P2K2 treatments. Forage total NSC yield ranged from 478 kg ha^{-1} for the FYM+N4P2K2 to 672 kg ha^{-1} for the FYM treatment. The highest forage WSC yield was observed for FYM whilst the lowest starch yield was obtained from the FYM+N4P2K2 treatment.

Table 3. Effect of fertilization treatments on evaluated lucerne stand traits (PD = plant density; SD = stem density; MSL = maximal stem length; LWR = leaf weight ratio; DMY = dry matter yield) in the first cut 2014.

Treatment	PD (m^2)	SD (m^2)	MSL (cm)	LWR (g kg^{-1})	DMY (t ha^{-1})
NIL	172 ^{b}	658 ^{a}	56 ^{a}	478 ^{c}	5.08
N4P2K2	187 ^{b}	966 ^{b}	72 ^{bc}	407 ^{ab}	5.27
FYM	168 ^{b}	691 ^{a}	68 ^{b}	424 ^{b}	5.47
FYM + N4P2K2	126 ^{a}	733 ^{a}	79 ^{c}	394 ^{a}	5.18
p	0.003	<0.001	<0.001	<0.001	0.733

n = 4; one-way ANOVA; different letters document statistical differences for Tukey HSD, α = 0.05.

Table 4. Effect of fertilization treatments on water-soluble carbohydrates (WSC) and starch hectare production in lucerne leaves, stems and whole forage in the first cut 2014.

Treatment	Leaves		Stems		Forage	
	WSC (kg ha^{-1})	Starch (kg ha^{-1})	WSC (kg ha^{-1})	Starch (kg ha^{-1})	WSC (kg ha^{-1})	Starch (kg ha^{-1})
NIL	115 ^{b}	149 ^{b}	339 ^{ab}	15.9 ^{b}	455 ^{bc}	165 ^{b}
N4P2K2	110 ^{b}	127 ^{b}	316 ^{a}	17.2 ^{b}	425 ^{ab}	144 ^{b}
FYM	128 ^{b}	135 ^{b}	397 ^{b}	12.6 ^{a}	525 ^{c}	147 ^{b}
FYM + N4P2K2	74.1 ^{a}	99.8 ^{a}	290 ^{a}	14.5 ^{ab}	364 ^{a}	114 ^{a}
p	<0.001	0.001	<0.001	0.004	0.001	0.001

n = 4; one-way ANOVA; different letters document statistical differences for Tukey HSD, α = 0.05.

The contributions of the fertilization and canopy traits to variability of lucerne NSC content are summarized in Table 5. In the first analysis A1, fertilization explained 57% of the variability in lucerne NSC content. Relationships among treatments and variables are illustrated in an ordination biplot after forward selection (Figure 2). The first (horizontal) canonical axis represents the mineral fertilization effect, showing treatments without mineral application on the left side of the figure. The mineral supply was related to lower WSC and starch content in association with higher stem length and leaf yield together with lower leaf weight ratio. The second (vertical) axis represents the specific response of mineral fertilization alone associated with higher leaf WSC and starch together with high stem density and low leaf weight ratio. In the A2 analysis, after exclusion of canopy traits as a covariate, the contribution of fertilization to the total effect was reduced to 29%. Along this standardization, a positive effect of the FYM treatment on leaf and forage WSC was observed with a negative correlation with stem starch. Forage and leaf starch were related to NIL and FYM+N4P2K2 treatments.

Table 5. Results of redundancy analyses investigating effects of explanatory variables on variability of WSC and starch content in lucerne leaves, stems and whole forage. Descriptions of canopy traits are given in Sections 2.3 and 2.4.

	Explanatory Variables	Covariate	% ax.	F-Test	*p*
A1	Fertilization	-	56.6	7.5	0.002
A2	Fertilization	Canopy traits	29.2	2.4	0.042

% ax.—adjusted variability of lucerne NSC explained by all canonical axes; F-test—F statistics for the test of all axes; *p*—corresponding probability value obtained by the Monte Carlo permutation test (499 permutations) for the test of all axes.

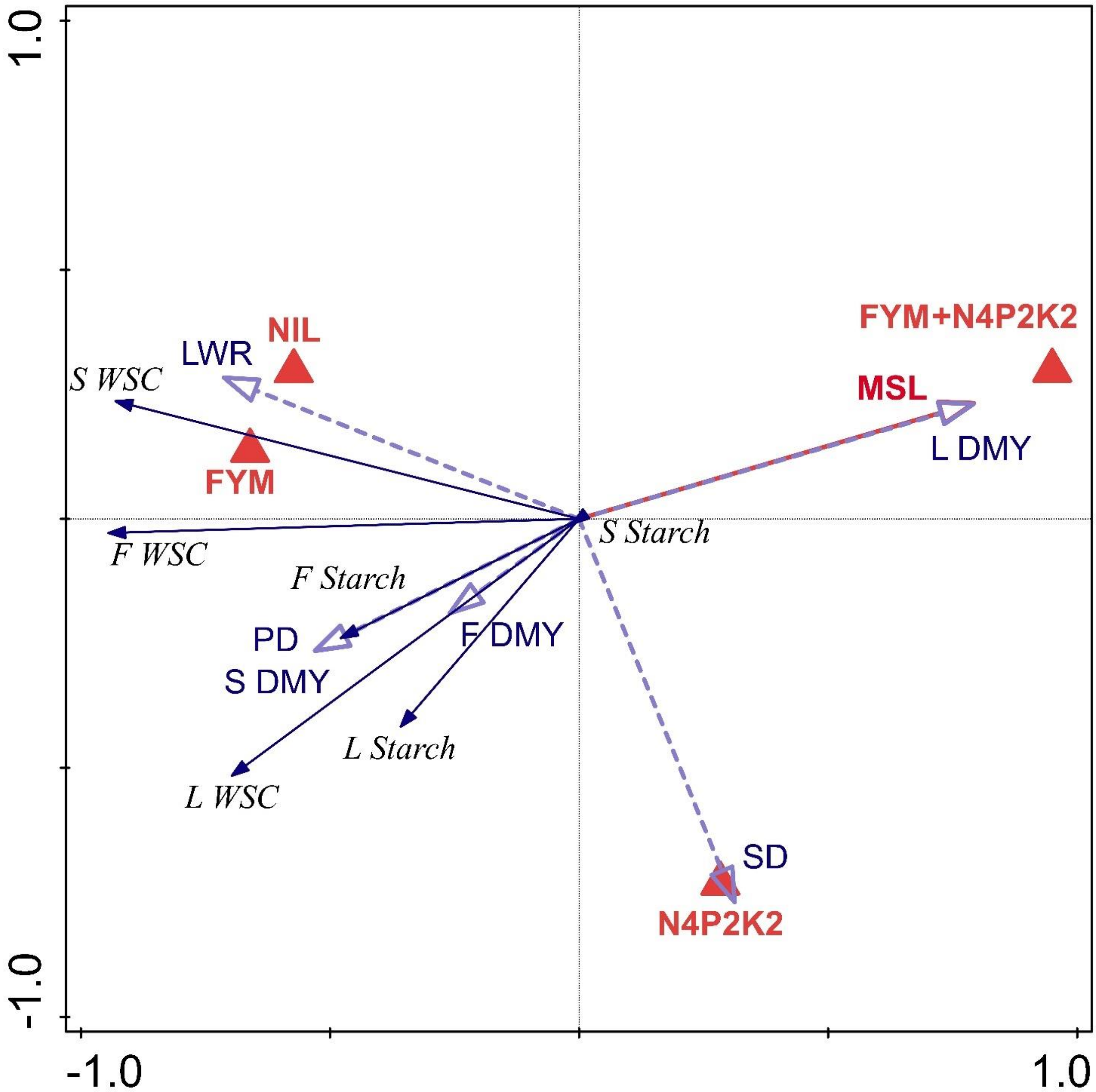

Figure 2. Ordination biplot showing the relationship between lucerne WSC and starch concentration (dependent variables, black solid line arrows) and explanatory variables considering fertilization treatments (triangle labels) and maximal stem length (red arrow) with respect to supplementary variables of canopy traits (broken line arrows). Abbreviations: DMY = dry matter yield of sample, F = variable related to whole forage, FYM = farmyard manure, L = variable related to leaf fraction, LWR = leaf weight ratio, MSL = maximal stem length of lucerne stem in the sample, NIL = unfertilized control, N4P2K2 = mineral fertilization, PD = plant density, S = variable related to stem fraction, SD = stem density, WSC = water-soluble carbohydrates.

4. Discussion

4.1. Effect of Fertilization on the NSC Content of Lucerne Herbage

This study demonstrated that application of mineral NPK fertilization to crops within a rotation affected only the WSC content, with only a limited effect observed on lucerne leaf WSC, and simultaneously reduced WSC content in stems, when compared with the FYM treatment or the unfertilized control. Although optimal P application could lead to higher soluble sugar content in lucerne [31], there was a negative effect of mineral NPK supply on NSC in the present study. This could be associated with a trade-off between structural and non-structural carbohydrates [27] or competition with increasing N compounds [26]. Enhancement of lucerne NSC content during the day is also usually accompanied by a decrease in N concentration and fiber fractions [16]. Comparison with previously reported changes in nutritive value of lucerne plant parts in the same experiment [8] suggests that the observed changes in WSC correspond more with differences in NDF than crude protein. The content of this was, unlike WSC, consistently lower in both lucerne parts and whole forage when control treatment NIL was compared with N4P2K2.

The positive effect of the manure-alone treatment on WSC accumulation in lucerne could be related to the long-term effects of manure on soil properties and consequently on plant growth, with this long-term management contributing to the development of a positive "soil environment effect" [8]. This phenomenon is difficult to demonstrate in short-term experiments where the effect of organic fertilizers on lucerne nutritive value has been related more to the direct effect of the applied nutrients [6], or even a zero effect, similar to the findings of a two-year study by Lestingi et al. [32] on the effects of digestate and organic mineral fertilizer. In previous work on the Ruzyně Fertilizer Experiment, Menšík et al. [33] showed that mineral NPK application without organic fertilizers was associated with degradation of soil quality, based on analyses of soil data from the last 25 years. Together with soil properties, manure fertilization also supports microbial populations [34] and development of arbuscular mycorrhizal fungi (AMF) [35–37]. These changes in soil microbiology could contribute to significant effects on lucerne forage quality as shown by the differences in WSC accumulation described in the work reported here. Baslam et al. [38] reported a positive impact of AMF inoculation on sucrose, glucose and fructose content in lucerne stems, although leaf content was not influenced. This finding is in line with the highest effect of manure on WSC enhancement in stems (and consequently in the whole forage). A similar positive effect of AMF inoculation on lucerne quality was also published for sugar content [31] and fatty acid profile [39]. An explanation could be that inoculation with AMF leads to increased chlorophyll content in the leaves and enhanced photosynthetic efficiency, and that it promotes absorption of P and enhances the resistance of plants to biological and abiotic stresses [40,41]. On the other hand, excessive P application has been shown to cause reduced AMF infection rate and lower soluble sugar content of lucerne [31]. This corresponds to the lower stem WSC content under the mineral application treatments in the present study (N4P2K2 and FYM+N4P2K2). As the diurnal balance of NSC accumulation and degradation has high relevance [16,17], further research on NSC daytime fluctuations could be valuable for better understanding of the role of the soil environment in these changes in plant chemical composition.

4.2. Contribution of Fertilization to Lucerne NSC through Canopy Structure Traits and Forage Yield

Fertilization treatments were reflected in differences in canopy structure, as treatments with higher nutrient supply had greater stem length and lower leaf weight proportion. This finding is in line with changes described by Hakl et al. [8]. Among canopy traits, forward selection in RDA showed that maximal stem length (together with leaf yield per m^2) was the driving factor in the negative correlation with WSC content in both plant part and leaf starch (Figure 2). It also corresponds with the suggestion of Peng et al. [27] about dilution effects of increased plant biomass following fertilization. Liu et al. [31] did not detect any correlation between lucerne plant biomass and soluble sugar contents. Similarly, Ruckle et al. [17] reported that biomass yield does not correlate with starch concentration in 128 red clover genotypes. Results summarized in Figure 2 confirm the minor role of forage yield for NSC accumulation but they highlight the importance of maximal stem length among canopy traits in this regard. It is also consistent with results where leaf weight proportion, with high impact on lucerne nutritive value, depends more on the stand height than on crop yield [42]. The present study suggests that some plant architecture traits could be more relevant to NSC than biomass accumulation per area unit. Liu et al. [31] found that the correlation between lucerne plant height and soluble sugars was non-significant in the pot fertilization experiment; however, their analysis used only simple Pearson's correlation coefficients and not multivariate methods.

A stable positive impact of regular manure applications, independent of changes in the canopy, can be demonstrated under standardized canopy traits (RDA, A2 in Table 5). This resulted in higher maximal stem length together with stem WSC content being maintained with regular manure applications, in comparison with the control NIL. This could be explained by the positive impact of AMF on lucerne sugar content, as discussed above [31,38], together with the benefit of manure on lucerne root accumulation [43], which resulted in reduced plant stress and improved stem NDF digestibility [8]. About 50% of the variability in lucerne NSC in relation to fertilization could be attributed to changes in forage yield and canopy characteristics. This 50% value is comparable to previous studies using multivariate analyses where 50% of the fertilization effect on forage quality could be explained by changes in canopy traits of lucerne stand [8] as well as by variability in yield and proportions of functional groups in grassland [44].

Forage yield was not significantly different between fertilization treatments, although positive lucerne yield responses to P and/or K application have been reported [3,9]. In line with this, a comparison over an 8-year period for this long-term experiment showed positive yield effects of manure and mineral fertilization on first cut as well as on annual forage yield, with the NIL treatment significantly lower than the other three treatments considered in this study [45]. Yield response to fertilization also depends on the temperature-precipitation relationship, in which more humid conditions may mask the effect of nutrient supply [8]. In contrast to the non-significant yield effects, there were differences in NSC hectare yield and these occurred in association with changes in plant part proportion and their NSC content. Hectare yield of starch and leaf WSC were reduced only in treatment FYM+N4P2K2, in contrast to other treatments. Stem WSC yield was reduced by both mineral treatments compared with the manure-only treatment. Similarly, forage WSC yield was highest for the FYM treatment, with the most significant reduction at FYM+N4P2K2. This demonstrates that lucerne stands providing the same yield can show contrasting effects in terms of canopy traits followed by variation of NSC content, thereby resulting in different hectare NSC yields. A question remains whether the NSC yield reduction observed for some treatments could be compensated for by significant yield enhancement reported over an 8-year period for this long-term experiment [45].

5. Conclusions

Our study provides evidence of residual effects on lucerne from long-term fertilization applied to previous crops. This was mainly reflected in the WSC content of lucerne, and intensive mineral fertilization consistently reduced WSC content in stem and in whole forage, compared with the unfertilized control treatment or that with manure alone. These changes in WSC content could be associated with dilution effect under the highest increase of maximal stem length for these treatments. Leaf WSC content was only reduced under the combined effect of manure and mineral fertilization. The manure treatment improved leaf and forage WSC relative to mineral fertilization alone despite the treatments having similar stem length and leaf weight ratio. In comparison with the unfertilized control, manure application maintained the same WSC in both stem and leaf fractions and in whole forage, in contrast to higher stem length and lower leaf weight ratio. This positive response could be attributed to improved soil conditions developing under regular manure application, which might include changes in soil microbiota, such as increased colonization with AMF and consequently reduced environmental stress; these aspects merit further study. A positive effect of manure was also visible in the hectare WSC yield despite no significant differences in forage yield. Fertilization explained 57% of the variability in NSC and this effect was halved after exclusion of canopy traits. This study has demonstrated clearly that manure fertilization of preceding crops in a rotation has potential for improvement of lucerne WSC, despite the possible negative relationship between lucerne stem length, which is promoted under higher nutrient supply, and forage NSC accumulation.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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