The $^{13}$C Discrimination of Crops Identifies Soil Spatial Variability Related to Water Shortage Vulnerability

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Abstract: Spatial variability of crop growth and yields is the result of many interacting factors. The contribution of these factors to variable yields is often difficult to separate. This work studied the relationships between the $^{13}$C discrimination ($\Delta^{13}$C) of plants and the spatial variability of field soil conditions related to impacts of water shortage on crop yield. The $^{13}$C discrimination, the indicator of water shortage in plants, $^{15}$N ($\delta^{15}$N) discrimination, and nitrogen (N) content were determined in grains of winter wheat, spring barley, and pea. The traits were observed at several dozens of grid spots in seven fields situated in two regions with different soil and climate conditions between the years 2017 and 2019. The principles of precision agriculture were implemented in some of the studied fields and years by variable rate nitrogen fertilization. The $\Delta^{13}$C significantly correlated with grain yields (correlation coefficient from 0.66 to 0.94), with the exception of data from the wetter year 2019 at the site with higher soil water capacity. The effect of drought was demonstrated by statistically significant relationships between $\Delta^{13}$C in dry years and soil water capacity ($r$ from 0.46 to 0.97). The significant correlations between $\Delta^{13}$C and N content of seeds and soil water capacity agreed with the expected impact of water shortage on plants. The $^{13}$C discrimination of crop seeds was confirmed as a reliable indicator of soil spatial variability related to water shortage. Stronger relationships were found in variably fertilized areas.

Keywords: precision agriculture; drought; soil water capacity; nitrate leaching; management zones

1. Introduction

1.1. Precision Agriculture

Precision agriculture (PA) is based on direct or indirect identification of the spatial variability of various factors, especially field soil conditions, affecting plant growth and yield formation, yield quality, or plant health. Various farm practices are adjusted with respect to the variability of these factors, historical yields, and actual crop status with the aim of increasing productivity and effectiveness of resources without negative impacts on the environment [1–4]. Spatial variability of crop growth and yields is the result of many interacting factors. The contribution of these factors to variable yields is often difficult to separate. Water is a key determinant of plant growth, and thus variable water content, as the result of soil texture and hydraulic properties, slope, etc., affects growth and yield [5–11].
Water shortage, i.e., low soil water availability in the root zone, affects most processes in soil, for example, nitrogen (N) mineralization, ion diffusion, and transport of water to roots. Drought often increases yield spatial variability and affects the efficiency of agronomic measures, including fertilization. Climatic trends in uneven precipitation distribution within a year, higher temperatures, and evapotranspiration result in soil-related vulnerability to water shortage becoming even more pressing \[12,13\].

Field (FWC) and available water capacity (AWC), together with the rate of infiltration, are listed among the most important indicators of soil spatial variability in farm fields. Soil texture, i.e., the proportions of sand/clay/silt particles, corresponds to FWC and AWC. These characteristics are used as proxies of soil hydropedological traits instead of laboratory determination of soil hydrolimits and water retention curves \[14\]. Furthermore, the different proportions of sand and clay correlate more or less with soil organic matter content and the availability of nutrients for crops. Thus, it may be difficult to distinguish the causes of the spatially variable impact of drought and nutrient availability \[10\].

1.2. The Impacts of Soil Spatial Variability on Water Regime and Water Quality

Intensive agricultural production, especially on arable land, is the main source of diffuse nitrate pollution and nitrates in water, which causes problems for water quality requirements \[15–19\]. Conventional agriculture applies uniform rates of N fertilizers across the whole field. A reduction of growth and yield and the resulting lower depletion of N due to unpredictable low precipitation and water shortage increase the amount of residual nitrate nitrogen left after the harvest that may be leached, especially during the autumn to spring period. Under irrigation, the soil variability may cause losses of nitrogen due to the percolation of water with nutrients and their leaching from the root zone as a result of the application of irrigation doses that do not correspond to the capacity of the soil to retain water \[20\].

Both regions of the Czech Republic (i.e., Bohemian–Moravian Highlands and lower Jizera River) where the research was carried out (Figure 1) are important zones of drinking water for more than a million inhabitants of Prague and other sites. At the same time, the areas belong to important agriculture regions, producing mainly potatoes, vegetables, and silage maize; all three crops are known for an increased risk of nitrate leaching \[20\], as well as oilseed rape and cereals.

The experimental sites in the Bohemian–Moravian Highlands belong to the Švihov drinking water reservoir basin on Želivka River with a catchment area of 1178 km\(^2\), which provides 75% of the drinking water for Prague. The arable soil in this region, which constitutes 75% of the agricultural land, is used for the production of cereals, silage maize, oilseed rape, and potatoes.

The other region, the area along the lower Jizera River (the Káraný water works), is the source of the other 25% of the total drinking water volume for Prague. Water is extracted by over 600 bore wells supplied by water seeping from the river and the percolation from adjacent fields \[16\]. The area is used for the cultivation of irrigated vegetables and early and medium potatoes. Monitoring of crop production and soil mineral N (NO\(_3\)-N + NH\(_4\)-N) content down to a 120-cm depth in the area showed high nitrate contents in the deep subsoil layers (under 60 cm), not accessible to the roots of many vegetables and potatoes \[21,22\]. This can be linked to the observed trend of increasing nitrate concentrations in extracted water in the last two decades. Soil vulnerability to leaching is amplified by high spatial variability of soil properties, which is manifested visually, by water stress impacts, when non-irrigated crops are grown in the fields.
reduce water consumption through transpiration, in turn leading to different concentrations of CO₂ inside the leaves, and to changes in the 13C and 12C isotopic ratio [25]. The discrimination of 13C in seeds integrates the effects of available water supply and plant water status during growth. The plants that sustain their stomata open, thanks to a greater available water supply or to the genotype’s effective water management, have a higher Δ13C. This suggests higher photosynthetic activity and a higher yield [26]. For example, Raimanová et al. found in field experiments that every 100 mm of water in precipitation and irrigation corresponded to an increase of Δ13C by approximately 1% in wheat grains [27]. Additionally, Δ13C of soil and its components have been utilized to study soil spatial variability [28,29]. Furthermore, the discrimination of 15N, together with 13C, may provide new information on the impact of abiotic stresses [30].

We hypothesize that under conditions of a high spatial variability of soil conditions and soil water capacity, yield differences are caused primarily by the impact of water stress. The discrimination of 13C isotope, which integrates water availability conditions during growth, should therefore correlate with both yield and soil water capacity, which determines the available water supply in the root zone. It can be expected that in a wetter locality or in rainier years, these relationships will be weaker.
The objectives of study were (1) to determine the relationship between the $^{13}$C discrimination of crop seeds and yield and (2) to relate the $^{13}$C discrimination to the spatial variability of soil water capacity at sites with different soil and climate conditions.

2. Materials and Methods

2.1. Experimental Sites

The experiments were carried out on farm fields in two regions with different soil and climate conditions (Figure 1 and Table A1).

2.1.1. Lower Jizera River Region

The four experimental fields, denoted So5, So7, and So9, near the village of Sojovice and Ko1 near Kochánky village in the lower Jizera River region were monitored between the years 2017 and 2019 (Figure 1a, Figure S1, and Table A1). Pea (Pisum sativum L.) and winter wheat (Triticum aestivum L.) were grown in the years 2017–2019 in Kochánky, while winter wheat and potatoes (Solanum tuberosum L.) were grown in the same years in Sojovice. Winter wheat was grown after pea and wheat (Kochánky) or after potatoes or wheat (Sojovice). The data for three fields in Sojovice were merged for correlation analysis as their soil conditions are similar. The soil and plants were sampled at the same grid points; in 2019, three spots were not sampled in Kochánky due to dividing the field for different crops. The field So7 in Sojovice was divided into two parts, A and B, where irrigated potatoes and non-irrigated wheat were grown alternatively.

2.1.2. Bohemian–Moravian Highlands Region

Three fields with winter wheat (Triticum aestivum L.) and a field with spring barley (Hordeum vulgare L.) were studied between the years 2017 and 2019 in the Bohemian–Moravian Highlands, near the villages of Kojčice (Koj), Svépravice (Sv), and Krasíkovice (Kr) (Figure 1b, Figure S1, and Table A1). Winter wheat was grown after oilseed rape (in 2017 and 2018) or after spring barley (2018). Spring barley (2019) was grown after potatoes. Precision agriculture principles were used in some areas of the field, i.e., mineral N fertilizers were applied in a spatially variable manner according to long-term crop yield potential (2017–2018) and the actual crop status derived from satellite images of Sentinel 2a and 2b (2018) (Figure S1). Other areas of the field were fertilized uniformly, i.e., without any respect to soil heterogeneity and its effect on crop water and nutrient uptake.

At both sites, the experimental crops were grown without irrigation.

2.2. Sampling and Soil Analysis

In the lower Jizera River region, the soil and plants were sampled in the same 21 (Ko1), 14 (So5), 8 (So7A), 9 (So7B), and 9 (So9) grid points selected according to aerial photos from the dry years 2015 and 2016 to represent the areas of different drought impact on wheat and sugar beet crops. In the Bohemian–Moravian Highlands region, the plants were sampled in 33 (Sv in 2017), 23 (Kr), 33 (Koj), and 53 (Sv in 2019) grid points. Soil samples were collected in a reduced number of points: 31 (Sv in 2017), 16 (Kr), 23 (Koj), and 26 (Sv in 2019).

The soil sampled at the grid points was analyzed for soil texture classes by the pipette method [31], which determined the particle size distribution in the topsoil (0–30 cm) and subsoil (30–60 cm) layers at both sites and in the deep subsoil (60–90 cm) in the lower Jizera River region.

A fine particle size fraction (FPSF, %) < 0.01 mm was used for calculating the FWC, the wilting point (WP), and the AWC with simple pedotransfer functions [32]:

\[
\text{FWC (vol.\%)} = 6.66 + 1.03 \times (\text{FPSF}) - 0.008 \times (\text{FPSF})^2, \tag{1}
\]

\[
\text{WP (vol.\%)} = 2.97 + 0.33 \times (\text{FPSF}) - 0.0012 \times (\text{FPSF})^2, \tag{2}
\]
AWC (vol.%) = FWC − WP. \hspace{1cm} (3)

The values of water capacity were reduced according to the stone content (>2 mm).

The above-ground parts of the plants were sampled from the two areas (0.25 m² in the lower Jizera River region or 0.2 m² in the Bohemian–Moravian Highlands region) near each grid point shortly before or at maturity (BBCH 87–90). The dry matter weight of grains was determined at both sites and the yields are presented in dry matter.

Water balance was calculated in both regions in each experimental year as the difference between the sum of daily precipitation and the sum of daily Penman’s evapotranspiration [33] during the growing season (Figures 2 and 3).

\[
\Delta^{13}C = \left(\delta^{13}C_a - \delta^{13}C_p\right)/\left(1 + \delta^{13}C_p(1000)\right), \hspace{1cm} (5)
\]

where $Rs$ is the ratio $^{13}C/^{12}C$ of the sample and $Rb$ is the $^{13}C/^{12}C$ ratio of the PDB.

For the description of changes in $C$ isotopes, the value of the $^{13}C$ discrimination was used:

\[
\delta^{13}C(\%o) = [(Rs − Rb)/Rb] \times 1000, \hspace{1cm} (4)
\]

The C isotope content of the seeds was determined using the elementary analyzer EA 3200 (Eurovector, Italy) coupled to the isotopic mass spectrometer Isoprime (GV Instruments, UK) at the Crop Research Institute, Prague. Plant material was dried and ground into a fine powder with a ball mill (Retch, Germany).

Crop Research Institute, Prague. Plant material was dried and ground into a fine powder with a ball mill (Retch, Germany).

Figure 2. Precipitation and water balance (precipitation minus evapotranspiration) in the years 2017–2019 in Brandýs n. Labem (near Sojovice).

Figure 3. Precipitation and water balance (precipitation minus evapotranspiration) in the years 2017–2019 in the Bohemian–Moravian Highlands region (2017 and 2019, Svépravice; 2018, Krasíkovice).

2.3. Plant Isotope Analysis

The $C$ isotope content of the seeds was determined using the elementary analyzer EA 3200 (Eurovector, Italy) coupled to the isotopic mass spectrometer Isoprime (GV Instruments, UK) at the Crop Research Institute, Prague. Plant material was dried and ground into a fine powder with a ball mill (Retch, Germany).

The value of the carbon isotopic ratio $\delta^{13}C$ was calculated as the ratio $^{13}C/^{12}C$ of a sample and of the PDB (Pee Dee Belemnite) standard:
where $\delta^{13}C_a$ is the $\delta^{13}C$ value of air (~8‰) and $\delta^{13}C_p$ is the measured value of the plant [26].

The content of N (%) and the content of N isotopes $^{15}$N and $^{14}$N were determined. The value of the $^{15}$N isotopic ratio ($\delta^{15}$N) was calculated as:

$$\delta^{15}\text{N}(%)= [(R_s - R_b)/R_b] \times 1000,$$

where $R_s$ is the ratio $^{15}$N/$^{14}$N of the sample and $R_b$ is the $^{15}$N/$^{14}$N ratio of the standard and the atmosphere air [34].

2.4. Statistical Analyses

Statistical data evaluation was performed using Pearson regression analysis (with a significance level of $p < 0.05$). Statistica 13 (Stat-Soft Inc., Tulsa, OK, USA) software and the R environment [35] were used.

3. Results

3.1. Lower Jizera River Region

The water balance in the experimental years showed a shortage of water for the main part of the growth season of pea and winter wheat (Figure 2). The precipitation sum from 1 March to 15 July amounted to 196 (42 mm on 29 May), 169 (50 mm on 9 June), and 125 mm in the years 2017, 2018, and 2019, respectively, nearby Brandýs nad Labem climatic station. The precipitation was insufficient to cover the water demand of pea and wheat; thus, the water supply from the soil reserves, which formed during winter and early spring, was an important source for sustaining plant growth. The water capacity of the soil within the root zone influenced the intensity of drought. These conditions strongly differentiated the growth and yields among the monitored points of the sampling grids.

The coefficients of variation (CVs) of the grain yield of pea in 2017 and of wheat in 2018 and 2019 in Kochánky were 55%, 32%, and 34%, respectively. The corresponding CVs of the wheat yields in Sojovice were 49%, 38%, and 50% (Table 1).

The strong spatial variability in yield corresponded to the variability of the FWC calculated from the soil texture. The FWC ranged from 21.9% to 31.4% in the top 0–30 cm of the soil and from 7.3% to 34.7% in the next 30–90 cm in Kochánky, and from 11.4% to 33.3% in the top 0–30 cm of the soil and from 8.7% to 34.7% in the next 30–90 cm in Sojovice (Table 1). The variability is equal to a difference of up to approximately 180 mm of water in the root zone (0–90 cm) of winter wheat. Soil moisture in early spring in the experimental fields showed that the soil was not filled to the FWC level; however, the maximum difference in the water content among spots of the same fields in spring reached up to 80–140 mm. The values of the FWC tightly correspond to the calculated values of the available water content.

The calculated FWC correlated significantly with the observed grain yields (Table 2). Similar relationships were observed between the total harvest biomass. The FWC explained from 58% to 78% of the variability in yield in the experimental years. The analysis showed significant relationships between the calculated FWC, the yield, and the $^{13}$C discrimination of the seeds ($\Delta^{13}$C), the indicator of water conditions during growth, at both sites and in all experimental years. The $\Delta^{13}$C showed a strong relationship with yield, i.e., correlation coefficients that ranged between 0.84 and 0.97. Except for one case, the correlation coefficients between $^{13}$C and the FWC were also above 0.80. The nitrogen content of the grains was negatively, mostly significantly related to the FWC, $\Delta^{13}$C, and yield. The $^{15}$N discrimination, $\delta^{15}$N, correlated positively with $\Delta^{13}$C, yield, and the FWC, and negatively with the N content of the grains. The corresponding correlations of the AWC were almost identical to the results of the FWC, due to a tight correlation between them.
Table 1. Median, mean, and coefficient of variation (CV, %) of the grain yield, the nitrogen (N) content (Ncont) of seeds, $^{13}$C ($\Delta^{13}$C) and $^{15}$N ($\delta^{15}$N) discrimination, and field (FWC) or available (AWC) water capacity. VAR, fertilized variably; UNI, fertilized uniformly.

| Site       | Year | Statistical Features | Yield (t ha$^{-1}$) | FWC (% vol.)  | $\Delta^{13}$C (%) | $\delta^{15}$N (%) | Ncont (%) | AWC (% vol.) |
|------------|------|----------------------|--------------------|---------------|----------------------|----------------------|-----------|-------------|
|            |      | All      | VAR | UNI | All      | VAR | UNI | All      | VAR | UNI | All      | VAR | UNI | All      | VAR | UNI | All      | VAR | UNI | All      | VAR | UNI |
| Svépravice | 2017 | Median   | 8.02 | 7.73 | 8.48 | 23.23 | 21.99 | 24.41 | 18.45 | 18.33 | 18.69 | 1.11 | 1.00 | 1.15 | 2.20 | 2.17 | 2.23 | 14.47 | 13.67 | 15.17 |
|            |      | Mean     | 8.12 | 7.68 | 8.59 | 23.14 | 21.97 | 24.39 | 18.40 | 18.29 | 18.53 | 1.17 | 1.09 | 1.26 | 2.20 | 2.15 | 2.25 | 14.37 | 13.66 | 15.13 |
|            |      | CV (%)   | 21.7 | 24.0 | 18.6 | 12.4  | 13.2  | 9.7   | 4.2   | 4.0   | 4.4   | 31.6 | 29.4 | 33.3 | 10.5 | 9.3  | 11.1 | 12.2 | 13.0 | 9.4  |
| Krasíkovice| 2018 | Median   | 6.63 | 7.44 | 6.61 | 19.58 | 21.39 | 19.12 | 18.04 | 18.04 | 18.01 | 1.93 | 1.86 | 2.05 | 2.46 | 2.49 | 2.45 | 12.18 | 13.31 | 11.88 |
|            |      | Mean     | 7.30 | 7.56 | 7.06 | 20.32 | 21.04 | 19.61 | 18.07 | 18.06 | 18.08 | 2.00 | 1.95 | 2.03 | 2.46 | 2.44 | 2.47 | 12.62 | 13.08 | 12.17 |
|            |      | CV (%)   | 27.3 | 33.6 | 19.4 | 14.8  | 15.0  | 14.6  | 3.1   | 3.5   | 2.9   | 40.5 | 33.8 | 47.8 | 6.9  | 7.4  | 7.3  | 14.7 | 14.8 | 14.6 |
| Kořice     | 2018 | Median   | 6.94 | 6.49 | 8.09 | 21.78 | 19.44 | 22.57 | 18.88 | 18.70 | 19.44 | 1.30 | 0.81 | 1.56 | 2.08 | 2.07 | 2.08 | 13.56 | 12.09 | 14.06 |
|            |      | Mean     | 7.25 | 6.51 | 7.94 | 21.10 | 19.51 | 22.83 | 18.95 | 18.68 | 19.21 | 1.37 | 0.94 | 1.77 | 2.11 | 2.13 | 2.10 | 13.10 | 12.10 | 14.17 |
|            |      | CV (%)   | 28.3 | 28.9 | 25.3 | 18.4  | 18.7  | 15.5  | 5.5   | 5.6   | 5.2   | 90.5 | 74.6 | 47.8 | 9.5  | 9.0  | 8.5  | 18.5 | 19.0 | 15.3 |
| Svépravice | 2019 | Median   | 6.49 | 6.68 | 6.88 | 24.26 | 21.16 | 21.9  | 2.41  | 1.72  | 1.72  | 2.19 | 2.41 | 1.74 | 1.74 | 1.74 | 1.74 | 14.55 | 12.5  |
|            |      | Mean     | 6.68 | 4.20 | 2.7  | 23.44 | 21.08 | 21.08 | 2.41  | 1.74  | 1.74  | 2.19 | 2.41 | 1.74 | 1.74 | 1.74 | 1.74 | 14.55 | 12.5  |
|            |      | CV (%)   | 22.0 | 12.7 | 3.0  | 12.7  | 3.0   | 12.7  | 3.0   | 12.7  | 3.0   | 12.7  | 3.0   | 12.7  | 3.0   | 12.7  | 3.0   | 12.7  | 3.0   | 12.7  |
| Kochánky   | 2018 | Median   | 6.65 | 6.65 | 6.65 | 23.91 | 16.58 | 3.64  | 3.83  | 14.85 |
|            |      | Mean     | 6.14 | 26.06 | 18.62 | 3.15  | 2.67  | 14.19 |
|            |      | CV (%)   | 31.8 | 21.9 | 7.8  | 21.9  | 7.8   | 21.9  | 7.8   | 21.9  | 7.8   | 21.9  | 7.8   | 21.9  | 7.8   | 21.9  | 7.8   | 21.9  | 7.8   |
| Sojovice   | 2019 | Median   | 7.45 | 6.96 | 6.96 | 23.77 | 20.09 | 0.45  | 2.80  | 14.85 |
|            |      | Mean     | 6.78 | 22.46 | 20.04 | 0.72  | 2.79  | 14.19 |
|            |      | CV (%)   | 34.1 | 27.2 | 2.4  | 27.2  | 2.4   | 27.2  | 2.4   | 27.2  | 2.4   | 27.2  | 2.4   | 27.2  | 2.4   | 27.2  | 2.4   | 27.2  | 2.4   |
|            | 2017 | Median   | 5.63 | 23.15 | 17.41 | 2.45  | 1.90  | 14.31 |
|            |      | Mean     | 6.86 | 22.12 | 17.94 | 2.60  | 2.06  | 13.55 |
|            |      | CV (%)   | 48.5 | 37.7 | 9.6  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  | 42.7  |
| Sojovice   | 2018 | Median   | 6.59 | 6.73 | 6.73 | 27.25 | 18.48 | 2.62  | 2.81  | 16.65 |
|            |      | Mean     | 6.73 | 26.06 | 18.44 | 2.66  | 2.78  | 15.93 |
|            |      | CV (%)   | 38.2 | 38.2 | 12.8 | 55.6  | 19.4  | 21.7  |
|           | 2019 | Median   | 5.96 | 5.74 | 5.74 | 20.42 | 19.25 | 2.58  | 2.33  | 20.42 |
|            |      | Mean     | 5.74 | 20.60 | 19.56 | 2.50  | 2.28  | 20.60 |
|            |      | CV (%)   | 49.8 | 49.8 | 49.8 | 49.8  | 6.3   | 55.2  | 14.0  | 41.4  |
Table 2. Correlation coefficients from linear regression analysis of the relationships between $^{13}$C ($\Delta^{13}$C), the N content of seeds (Ncont), the grain yield, and the field water capacity (FWC). VAR, fertilized variably; UNI, fertilized uniformly.

| Site         | Year | $\Delta^{13}$C × Yield | Ncont × $\Delta^{13}$C | FWC × Yield | Ncont × FWC |
|--------------|------|-------------------------|-------------------------|-------------|-------------|
|              |      | All VAR | All UNI | All VAR | All UNI | All VAR | All UNI | All VAR | All UNI | All VAR | All UNI |
| Svépravice   | 2017 | 0.76 *  | 0.66 *  | 0.46 *  | 0.18     | 0.54 *  | 0.55 *  | 0.48    | −0.61    | −0.39    | −0.88 * | −0.23    | −0.48    | −0.32    |
|              | 2018 | 0.88 *  | 0.94 *  | 0.87 *  | 0.58     | 0.84 *  | 0.99 *  | 0.67    | −0.80    | −0.74    | −0.89 * | −0.63    | −0.84    | −0.41    |
|              | 2019 | 0.94 *  | 0.93 *  | 0.97 *  | 0.52     | 0.68 *  | 0.69 *  | 0.54    | −0.81    | −0.86    | −0.77 * | −0.21    | −0.32    | 0.06     |
| Krasíkovice  | 2017 | 0.24    |         |         |          | 0.64 *  |         |         | −0.78    |         |         | 0.11     |
|              | 2018 | 0.89 *  |         |         |          | 0.88 *  |         |         | −0.78    |         |         | −0.69    |
|              | 2019 | 0.88 *  |         |         |          | 0.97 *  |         |         | −0.36    |         |         | −0.34    |
|              | 2018 | 0.93 *  |         |         |          | 0.91 *  |         |         | −0.78    |         |         | −0.60    |
| Kojčice      | 2017 | 0.84 *  |         |         |          | 0.74 *  |         |         | −0.50    |         |         | −0.34    |
|              | 2018 | 0.94 *  |         |         |          | 0.79 *  |         |         | −0.98    |         |         | −0.72    |
|              | 2019 | 0.90 *  |         |         |          | 0.82 *  |         |         | −0.48    |         |         | −0.48    |

* Significant at the 0.05 level.
3.2. Bohemian–Moravian Highlands Region

Similarly to the lower Jizera River region, the water balance during the growing periods (from 1 April to 31 July) in 2017–2019 mainly revealed a water deficit, i.e., non-fulfillment of crop water requirements. The precipitation sum from 1 April until 31 July amounted to 328 (37 mm on 19 July), 117, and 269 mm (38 mm on 22 May) in the years 2017, 2018, and 2019, respectively (Figure 3). The spatial variability of the soil was demonstrated by a large range of FWC in the topsoil 0–30-cm layer (Sv, 19.5%–31%; Koj, 14.2%–27.5%; Kr, 19.4%–26.6%), and in the subsoil 30–60-cm layer as well (Sv, 12.1%–28.6%; Koj, 11.6%–29.6%; Kr, 13.7%–28.3%) (Table 1), which resulted in a difference in soil water supply in terms of the FWC in the layer 0–60 cm up to 102 mm (Sv), 125 mm (Koj), and 95 mm (Kr). At the beginning of the growing season (late March/early April) in 2017 and 2019, the topsoil moisture content (0–30 cm) reached only 45%–64% of the FWC. Contrarily, at the beginning of the growing season in 2018, the topsoil was sufficiently and even excessively saturated by water (77%–128% of the FWC).

In 2017 and especially in 2018, when low sums of precipitation in May and June (73 and 69 mm, respectively) worsened yield formation, the significant relationships between grain yield and the FWC in the top 0–60 cm of the soil with $\Delta^{13}C$ were revealed ($r = 0.46–0.99$; Table 2). In the wetter year 2019, the grain yield was not affected by water deficit and, therefore, the correlations associated with $\Delta^{13}C$ were not significant (Table 2). However, a significant dependence of the grain yield on the FWC was proved in all years ($r = 0.54–0.84$; Table 2). The CV values of the grain yield proved higher spatial variability at sites with a lower FWC (i.e., Kr and Koj) in the very dry year 2018 (27.2% in Kr and 28.2% in Koj) compared to the site with a higher FWC (Sv) in the years 2017 (21.7%) and 2019 (22.0%) (Table 2), when water deficit was substantially lower compared to the year 2018.

In the years 2017–2018, some areas of the field had mineral N fertilizers applied variably (VAR), according to PA principles, which revealed stronger and significant dependence of the grain yield on the FWC and $\Delta^{13}C$ in comparison to the areas that were fertilized uniformly (UNI) (Table 2). The relationships of the grain yield and $\Delta^{13}C$ associated with the FWC were not significant. In the very dry year 2018, variable mineral N doses correlated significantly with $\Delta^{13}C$ (Kr $r = 0.88$, Koj $r = 0.62$) compared to the year 2017, when winter wheat was grown at the site with a higher FWC (Sv $r = 0.27$).

Similarly to the lower Jizera River region, the nitrogen content of the grains was negative, mostly significantly related to the FWC, $\Delta^{13}C$, and yield. The $^{15}N$ discrimination, $\delta^{15}N$, correlated positively with $\Delta^{13}C$, yield, and FWC, and negatively with the N content of the grains, with the exception of the wet year 2019 in Svépravice.

4. Discussion

The dry weather in the lower Jizera River region contributed, together with a light and permeable soil, to a pronounced manifestation of soil variability in plants in all years. Strong differences in plant height, growth, the time of onset and the severity of water stress, withering, and drying were distinguishable in fields at the scale of ≤1 m. This proves the notion the plant itself is the best indicator of soil conditions and fertility. Our results and a vast amount of data in the literature show that cereals, especially wheat, are deeply rooted and are able to effectively exploit water from zones down to 80–100 cm and even deeper [22,36,37]. As a result, plant growth and yield integrate various soil characteristics with the root zone in interaction with weather and agronomic factors [5]. Furthermore, the effect of soil conditions is intensified by a lower content of available nutrients in soil with higher proportions of sand and stones in comparison with more silty soils.

In the Bohemian–Moravian Highlands region, the years 2017 and especially 2018 were warm and dry, while the higher precipitation in 2019 improved the agro-meteorological water balance, and thus the crop growth and yield formation were determined by other soil-related factors. Spring barley plants (grown in 2019) benefited from the greater amount of precipitation in May (Figure 3), an important period for the formation of yield (number of fertile tillers and grains per year). Spring barley is known to have shallower roots and to be more susceptible to worse soil conditions than wheat, which has a
longer growth period and a greater root system [22]. The lack of a relationship between yield or soil water capacity and $\Delta^{13}C$ in barley is unlikely attributed to a better ability of barley to withstand water shortage, and the observed significant variability in yield was caused by other spatially variable factors.

Except for spring barley in 2019, the $\Delta^{13}C$ explained from 58% to 95% of the yield variability in the lower Jizera River and Bohemian–Moravian Highlands regions, which was always better than relating the yields to the FWC (29%–77%). The better performance of $\Delta^{13}C$ could be explained by integrated soil and water conditions of the plants in the sampled areas. Most studies found a significant relationship between the $\Delta^{13}C$ of the leaves or seeds and yield under different water supplies (e.g., [27,38]); however, there is a lack of work on the spatial variability of the $\Delta^{13}C$ in field crops. According to Clay et al., the $\Delta^{13}C$ of soya seeds explained 62% of the total yield variability [11]. However, the authors noted that $\Delta^{13}C$ can be used to help assess water stress, provided that N stress is absent. Similarly, Clay et al. suggested that $\Delta^{13}C$ provides an index for water stress under non-nutrient-limiting conditions [10]. The data presented herein came from experimental fields fertilized with optimal rates of nutrients; however, visual signs of nutritional problems could be observed in a few spots with sandy and stony soils at the Kochánky and Sojovice sites.

As expected, more results could be found on the spatial variability of soil water-related traits in relation to crop growth and yield. Wong and Asseng found, under Mediterranean climate conditions, linear relationships between grain yield and the plant available water storage capacity (PAWc) of the top 100 cm of the soil profile in a 70-ha field [39]. Unlike our results, the authors found that spatial variability was reduced in low rainfall years when yields across the field were low and the higher soil water storage capacity of some sites was often underutilized. With adequate N, spatial variability increased with seasonal rainfall at sites with higher PAWc, which conserved more water in wet seasons to provide a higher yield response than sites with low PAWc. Under the transitional (maritime–continental) climate conditions of the Czech Republic, the situation is different; soil water supply is always at least partially replenished during the autumn–spring period. Unlike many other authors, Castellini et al. found a negative correlation between PAWc and durum wheat yield under Mediterranean climate conditions, probably because the soil parameters were determined only to a 10-cm depth [8]. Lipiec and Usowicz [40] reported that, under the wetter and colder conditions of central Poland, cereal grain yields were significantly negatively correlated with the sand content in the top- and subsoil in one of three experimental years, while Usowicz and Lipiec reported a positive correlation between cereal yields and topsoil water or subsoil clay contents [9].

The FWC and AWC are not the only traits that determine the water dynamics and availability for plants [8]. For example, the effect of other factors, such as the tracks of farm machines, may influence soil water parameters [41,42]. We observed similar significant correlations between $\Delta^{13}C$, FWC, pea, or wheat yields and the simple index derived from aerial RGB images taken from the air during sugar beet and wheat growth in the years 2015 and 2016 (previous to the experimental ones) in fields in Kochánky [43]. This showed the conservative character of soil traits connected with vulnerability to drought.

The impact of different soil water capacities under low precipitation during growth could be expected. The soil texture, especially the clay content (proportion), also affects nutrient content. The correlations between grain yield and the content of Ca and Mg in the top- and subsoil layers were mostly significant ($r = 0.4–0.7$) thanks to the relationship between the nutrients and the texture and humus content. Further, Müller proposed that an increasing content of clay functions as a “lubricant” for root growth, while a high content of sharp sand particles can damage root tissues [44]. Little is known about the effect of soil spatial variability on root size and depth. Kirkegaard et al. found an additional 10.5 mm of subsoil water uptake from the 1.35–1.85-m soil layer after the flowering stage, leading to a yield increase of 0.62 t ha$^{-1}$ in wheat [45]. We observed shallower roots of wheat in zones with sandy soil and a high proportion of stones (soil particles > 2 mm) in comparison to spots where the proportion of silt and clay was higher. Deeper roots provide access to a larger supply of water in the subsoil and vice versa; thus, the impact of soil texture on the root system may contribute to spatial
differences, resulting in variable growth, yield, and $^{13}$C discrimination. As a further example, a higher number or better stability of biopores after pre-crop roots or earthworms and other soil organisms in medium and heavy soils may contribute to root growth and penetration into the deeper layers of the subsoil [46].

The experimental sites in the Bohemian–Moravian Highlands were selected because of their colder and wetter climate conditions in comparison to the lower Jizera River region. However, the climate has been changing during recent decades, and shorter or longer periods of low precipitation and above average temperatures, resulting in higher evapotranspiration, are becoming common even in highland regions. Similar results as in the drier and warmer region of the lower Jizera River, in terms of the significant relationships among $^{13}$C discrimination, yield, and soil water capacity, confirm that the water supply has become a key condition for high yields in the region [12,13]. The more frequent occurrence of water shortages will demand new approaches to farm management.

Low soil water capacity and vulnerability to drought inevitably result in an increased loss of nutrients, especially nitrate leaching. The risk of nitrogen losses is enhanced by worsened growth and yield, leading to lower utilization of N from conventionally applied fertilizers and a higher residual nitrate content [5]. Both experimental regions are known for a higher risk of nitrate loss by leaching [16,21,47,48] and both are included in the nitrate-vulnerable areas that encompass 49% of the agricultural soils in the Czech Republic. The adoption of precision agriculture approaches, including precision irrigation [49], respecting the spatial variability of soil water capacity and the vulnerability to drought, may significantly improve the nitrogen efficiency of crops [50].

Furthermore, the results of Dalal et al., i.e., the linear relationship between $\Delta^{13}$C in grain and the nitrogen utilization efficiency in wheat, suggest an interrelated effect of nitrogen nutrition and water availability [51]. We observed mostly significant correlations between the $\Delta^{13}$C of grains or yield and the nitrogen content of grains. This suggests that under water shortages, N is not effectively utilized for grain production, as the nitrogen was not diluted by growth. This was confirmed by a negative correlation between N content and the FWC. The observed negative relationships between N concentration and the $\delta^{15}$N of grains agree with the lower utilization of N from mineral fertilizers with a lower $\delta^{15}$N signature than N depleted from the soil supply (derived from organic fertilizers and soil organic matter) due to water shortage [52,53]. The lower values of $\delta^{15}$N are likely also caused by lower soil N supply from the mineralization of soil organic matter in lighter, sandier, and stonier field spots.

5. Conclusions

The monitoring of several fields confirmed the working hypothesis about the $^{13}$C discrimination. The impact of water stress on plant yield reliably indicated spatially variable soil conditions related to soil vulnerability by water shortage. The effect of water shortages was confirmed by correlations among yield, $^{13}$C discrimination, and soil water capacity. The hypothesis about weaker relationships of $\Delta^{13}$C under higher precipitation corresponded to results of spring barley in the year 2019. The soil vulnerability to water shortages also indicates a spatially specific higher risk of nitrate leaching. The adoption of precision agriculture approaches may contribute to both the adaptation to climate change-induced water shortage episodes and to reducing the nutrient leaching in spatially variable soils. The results of this study suggest that drought will play a major role in precision nitrogen management due to precipitation fluctuations and the deterioration of the water balance. The $^{13}$C discrimination of crops will help differentiate and identify the main causes of spatial variability as a necessary basis for application of relevant agronomic measures. A proven approach makes it possible to integrate immediately vulnerable zone maps into the precision farming system.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/11/1691/s1: Figure S1. Aerial photos showing the NDVI variability of the experimental fields with the sampling point grids.

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J.H., R.D. and P.F.; visualization, P.F. and G.K.; supervision and project administration, R.D. and J.H.; funding acquisition, R.D. All authors have read and agreed to the published version of the manuscript.

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Appendix A

Table A1. Overview of the conditions of the experimental sites (Czech Republic).

| Site        | Coordinates                | Average Yearly Temperature (°C) | Sum of Precipitation (mm) | Soil Type [54] (Spatially Variable)                                                                 | Soil Texture (Spatially Variable)                                      | Field | Exp. Years | Crop       |
|-------------|----------------------------|---------------------------------|---------------------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|-------|------------|------------|
| Kochánky    | 50.2757078N 14.7926503E    | 9.4                             | 515                       | Eutric Loamic Fluvisol, Leptic Arenic Fluvisol, Leptic Arenic Regosol, Skeletic Arenic Regosol, and Haplic Phaeozems | Sandy loam, sandy with skeleton, and loam                              | Ko1   | 2017       | Pea W. wheat |
| Sojovice    | 50.2139350N 14.7571592E    | 9.2                             | 522                       | Eutric Loamic Fluvisol, Leptic Arenic Fluvisol, Leptic Arenic Regosol                                | Sandy loam, sandy with skeleton, and loam                              | So5   | 2017       | W. wheat    |
| Svépravice  | 49.5046033N 15.2329764E    | 8.2                             | 721                       | Eutric/Dystric Cambisol, Eutric/Dystric Stagnosol, Leptic Cambisol                                   | Topsoil: Loamy sand and sandy loam Subsoil: Sandy and loamy sand with skeleton | Sv    | 2017       | W. wheat    |
| Kojčice     | 49.4678526N 15.2488619E    | 8.2                             | 721                       | Eutric/Dystric Cambisol, Eutric/Dystric Stagnosol, Leptic Cambisol                                   | Topsoil: Sandy, loamy sand, and sandy loam Subsoil: Sandy and loamy sand with skeleton | Koj   | 2018       | W. wheat    |
| Krasíkovice | 49.4580347N 15.2135450E    | 8.2                             | 721                       | Eutric/Dystric Cambisol, Eutric/Dystric Cambisol (Arenic), Leptic Cambisol, Cambic Leptosol, and Eutric/Dystric Stagnosol Cambisol | Topsoil: Loamy sand and sandy loam Subsoil: Sandy and loamy sand with skeleton | Kr    | 2019       | W. wheat    |

All sites belong to climate regime Dfb: humid continental climate, according to Köppen-Geiger classification [55].

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