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Do Soil Warming and Changes in Precipitation Patterns Affect Seed Yield and Seed Quality of Field-Grown Winter Oilseed Rape?

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Abstract: Increasing air and soil temperatures and changes in precipitation patterns as consequences of climate change will affect crop production in agricultural ecosystems. The combined effects of soil warming and altered precipitation on the productivity and product quality of oil crops are not yet well studied. Winter oilseed rape (OSR) (*Brassica napus* L., cv. Mercedes) was field-grown under elevated soil temperature (+2.5 °C), reduced precipitation amount (−25%), reduced precipitation frequency (−50%) both separately and in combination in order to investigate effects on crop development, seed yield, and seed quality. Soil warming accelerated crop development during early plant growth and during spring. At maturity, however, plants in all treatments were similar in quantitative (aboveground biomass, seed yield) and qualitative (protein and oil content, amino acids, fatty acids) parameters. We observed the long-term effects of the precipitation manipulation on leaf size, leaf senescence and biomass allocation. Seed yield was not affected by the altered climatic factors, perhaps due to adaptation of soil microorganisms to permanent soil warming and to relatively wet conditions during the seed-filling period. Overall, OSR performed well under moderate changes in soil temperature and precipitation patterns; thus, we observed stable seed yield without negative impacts on nutritive seed quality.

Keywords: climate change; altered precipitation patterns; soil warming; seed yield; seed quality; oilseed rape

1. Introduction

Climate change poses a challenge for crop production in the twenty-first century. Changes in temperature and precipitation patterns can result in either positive or negative effects on crop development and physiological plant processes, with impacts on crop yield and the chemical composition of seeds [1]. Under global warming, the mean air temperature in Germany is predicted to increase by 1.2–5.3 °C by 2100 compared to 1971–2000 [2]. Elevated air temperature will lead to an increase in soil temperature [3], affecting soil moisture content and crop development in agroecosystems [4]. In temperate climates it has been observed that the shoot growth of crops is limited by low soil temperature during early development stages [5]. Accordingly, even a small increase in soil temperature can accelerate crop growth and promote the development of crops [4,6]. Also, the uptake of water and nutrients from the soil and most likely the overall development of crops is accelerated due to elevated soil temperature [5].

Besides changes in soil temperature, amount and frequency of precipitation are also expected to be altered within coming decades in Germany [2]. Thus, water availability in agricultural areas will be affected, reflected by changes in soil moisture content. In agroecosystems, a reduction in water availability of crops can negatively affect harvestable yield [7,8], resulting in a major limitation to food production [9].

In Central Europe, *Brassica napus* L. is used both as an oil and a protein crop, with the winter variety most frequently cultivated due to its higher yields in comparison to summer varieties [10]. Winter oilseed rape (OSR) is predominantly cultivated in France and Germany [11] and is a preferred pre-crop for cereals due to its deep rooting system, which improves soil structure. The yield of oilseed rape is known to be highly variable, depending on temperature and precipitation conditions during the growing period. For instance, yield losses of winter oilseed rape appear to be correlated with rising mean temperatures [12]. Also, a deficiency in water availability occurring in the period from flowering to the end of seed set was found to reduce the seed yield of oilseed rape [13]. Thus, yield stability of oilseed rape will be a major challenge under changing climatic conditions in coming decades [12].

The seed quality of OSR is represented by the concentrations of oil, protein, and glucosinolates, all of which are expected to change under elevated temperature and water shortage [1]. In pot experiments with OSR, water stress induced by withholding of irrigation at different crop developmental stages [14] and reduction in water amount [13] increased the concentration of protein in seeds, but decreased oil concentration. Similar results were observed under elevated air temperature in soybean [15,16]. Under a water shortage, OSR accumulated secondary metabolites such as glucosinolates in mature seeds [17]. The utilization of OSR meal, which is a byproduct of oil extraction and a protein source for livestock, can be compromised by high glucosinolate content, due to the potential of glucosinolates to harm animals, e.g., leading to a reduction in milk production or to impaired reproductive activity [18].

Crops in agricultural landscapes will likely be exposed to several climate change factors at the same time. Hence, we investigated in the Hohenheim Climate Change (HoCC) experiment the interactive effects of soil warming and altered precipitation patterns on the yield and yield quality traits of OSR. In this temperate agroecosystem, an increase in soil temperature by 2.5 °C and, during summer months, a reduction in precipitation amount (−25%) and frequency (−50%) were simulated. Crop development was measured over the entire growing period. Seed yield data from three specific time points (stem elongation, flowering, maturity) and quality components of mature seeds were analyzed. The hypotheses were as follows: (i) soil warming will accelerate crop development only during spring but not afterwards; (ii) a reduction in precipitation amount during summer months will increase the protein concentration of the seeds, but reduce oil concentration; (iii) a reduction in precipitation frequency during summer will increase periods of reduced water availability, which will reduce the seed-filling period and thus decrease seed yield; and (iv) a simultaneous occurrence of soil warming, reduced precipitation amount, and precipitation frequency will have additive negative effects on seed yield.

2. Materials and Methods

2.1. Site Description

This study was conducted at the HoCC experimental research station (University of Hohenheim, Stuttgart, Germany), which was established in 2008 in order to investigate climate change effects on agroecosystems. The HoCC experiment is located at the Heidfeldhof research station (48°43′ N, 9°13′ E, 401 m a.s.l.). From 1961 to 1990, annual mean air temperature was 8.7 °C and annual precipitation was 679 mm [19]. In 2016 and 2017, when OSR was grown at the experimental site, the mean annual air temperature was 10.1 °C and 10.2 °C and annual precipitation was 595.4 mm and 830.9 mm, respectively (weather station "Hohenheim", [20]) (Figure 1). The soil at the field site is a loess-derived stagnic Luvisol of a pH 6.9, organic carbon content of 12.1 g kg^{-1}, and soil texture of 9.4% sand, 68.1% silt, and 22.6% clay [21].

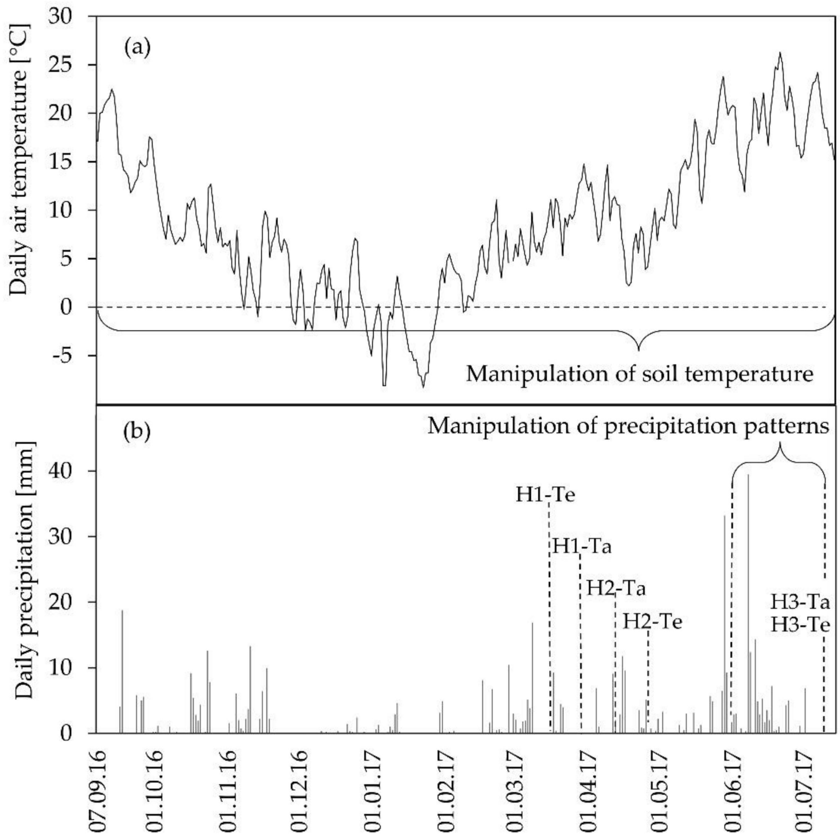

Figure 1. (**a**) Average daily air temperature at 2 m height and (**b**) daily precipitation at the experimental site during the growing season of winter oilseed rape (OSR) from 7 September 2016 to 11 July 2017. In total, three harvests were made: harvest 1 (H1) at stem elongation, harvest 2 (H2) at full flowering and harvest 3 (H3) at maturity. At the first and second harvests, plants grown under elevated soil temperature (Te) were harvested about two weeks earlier than plants grown under ambient soil temperature (Ta). Harvest dates: H1-Te, 15 March 2017; H1-Ta, 27 March 2017; H2-Te, 10 April 2017; H2-Ta, 24 April 2017. At maturity, plants grown under Ta and Te were similarly developed and harvested on the same day: H3-Te and H3-Ta, 11 July 2017. Data are from the weather station "Hohenheim" of the Agricultural Technology Centre (LTZ) Augustenberg, Germany. The manipulation of the precipitation patterns (−25% precipitation amount, −50% precipitation frequency) started on 6 June 2017, when plants had developed to between the middle of fruit development (development stage DC 76) and the beginning of ripening (DC 80) stage [22]. OSR was watered until plant maturity (H3).

2.2. Experimental Setup

Since 2008, soil temperature (T) as well as precipitation amount (A) and frequency (F) have been manipulated within the HoCC experiment, based on climate change predictions until 2100 for southwest Germany [23]. The treatments are replicated in four blocks in a split-plot design. Treatments are specified in Table 1. Each block is separated into two mainplots each with two 1 m × 4 m plots. Each plot is further split into four 1 m x 1 m subplots. Soil warming is simulated in one of the mainplots per block using heating cables located on the soil surface (RS 611-7918, RS Components GmbH). Soil temperature is elevated by 2.5 °C (Te) at 4 cm depth. Dummy cables on ambient temperature mainplots (Ta) are installed to account for side effects of the presence of heating cables, such as retention of water from precipitation. In one plot within each mainplot, a roof is used to manipulate precipitation (Folitec UV 5 foil, folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany). To manipulate subplots individually, each subplot is surrounded by a polyvinyl chloride (PVC) barrier to prevent lateral water movement. The 32 roof-covered subplots are watered manually with rainwater collected in rain barrels. During the period of the precipitation treatment, from June to August, the ambient precipitation amount (Aa) is reduced by 25% (Ar). Precipitation frequency simulates longer dry periods by reduction in the number of rainy days by 50% (Fr) as compared to ambient precipitation frequency (Fa) (i.e., the cumulative precipitation of two ambient rain events is delivered as one event). In the study period, alterations in precipitation patterns were simulated during the summer months from 6 June 2017 until plant maturity (11 July 2017). Soil temperature is recorded using temperature probes at 4 cm depth. TDR (time-domain reflectometry) probes (CS630/CS635, Campbell Scientific Ltd., Loughborough, UK) installed at 0–15 cm depth are used to measure soil moisture content. The combination of ambient soil temperature, ambient precipitation amount, and ambient precipitation frequency simulated under the roofs was taken as control conditions. The subplots are covered by roofs (roofed: R1), except that two subplots in each block have no roofs (roof-control: R0) in order to identify possible roof effects. Precipitation patterns are not manipulated in the roof-control plots. Additional information about the experimental setup can be found in Poll et al. [21].

Table 1. Treatment combinations of the experiment: ambient (Ta) or elevated (Te) soil temperature in combination with the following precipitation patterns: ambient (Aa) or reduced (Ar) precipitation amount and ambient (Fa) or reduced (Fr) precipitation frequency. In treatments 1–8 oilseed rape (OSR) was grown under roofs (R1) to enable the manipulation of precipitation patterns. OSR in treatments 9 and 10 was not covered by roofs (R0), to check for a roofing effect. Abbreviations: AMB = ambient; ELE = elevated; RED = reduced; temp. = temperature; prec. = precipitation.

Treatment Number	Roof	Treatment Description	Treatment Short Form
1	Yes	AMB soil temp. × AMB prec. amount × AMB prec. frequency	TaAaFa
2	Yes	AMB soil temp. × AMB prec. amount × RED prec. frequency	TaAaFr
3	Yes	AMB soil temp. × RED prec. amount × AMB prec. frequency	TaArFa
4	Yes	AMB soil temp. × RED prec. amount × RED prec. frequency	TaArFr
5	Yes	ELE soil temp. × AMB prec. amount × AMB prec. frequency	TeAaFa
6	Yes	ELE soil temp. × AMB prec. amount × RED prec. frequency	TeAaFr
7	Yes	ELE soil temp. × RED prec. amount × AMB prec. frequency	TeArFa
8	Yes	ELE soil temp. × RED prec. amount × RED prec. frequency	TaArFr
9	No	AMB soil temp. × AMB prec. amount × AMB prec. frequency	TaAaFa
10	No	ELE soil temp. × AMB prec. amount × AMB prec. frequency	TeAaFa

2.3. *Plant Cultivation, Crop Development Measurement, Biomass Harvests and Seed Quality Analyses*

Winter OSR (*Brassica napus* cv. Mercedes) was cultivated from 7 September 2016 to 11 July 2017. In total, 85 plants m^{-2} were sown and adjusted to a final density of 60 plants m^{-2}. Pre-crop was spring barley (*Hordeum vulgare* cv. RGT Planet). Plants were fertilized with 50, 60 and 40 kg N ha^{-1} calcium ammonium saltpeter on 28 September 2016, 7 March 2017, and 14 March 2017, respectively. OSR was weeded regularly to keep weed pressure low, snail granule was scattered when plants were small and insecticides were applied for chemical disease control (Supplementary Materials Table S1). In the center of each subplot, five plants were labelled and used to measure crop development parameters, which were measured weekly by using the BBCH decimal code (DC) [22].

Three harvests were performed at fixed crop developmental stages: (1) at the beginning of stem elongation (DC 31), (2) at full flowering (DC 65), and (3) at maturity (DC 99) (Table 2). At harvests 1 and 2, two representative plants per subplot were cut 1 cm above the soil surface and separated into green and senescent leaves, stems and flowers. Fresh and dry weights were determined. Green leaf area was determined with a leaf area meter (LI3000, Li-Cor, Lincoln, NE, USA) and specific leaf area (SLA) was calculated (green leaf area divided by green leaf dry weight). Leaves and stems were dried at 60 °C to constant weight, while flowers were dried at 30 °C. At harvest 3, all plants in a center 0.5 m × 0.5 m of each subplot were cut and separated into straw (stems and leaves) and pods. Pods were manually threshed in order to separate seeds and seed yield was measured. For the calculation of the harvest index (HI), seed yield per plant was divided by total aboveground biomass per plant (straw and seeds). Thousand seed weight was determined using a seed counter (Condator "E", Pfeuffer, Germany) [24]. Before quality assessment, seeds were milled into fine powder with a Brabender Quadrumat Junior mill. Total protein concentration was determined by the Kjeldahl method [25] and total oil concentration was analyzed by method H of the same regulation [26]. The composition of amino acids was analyzed using the European Commission Regulation (EC) No 152/2009 III F [27], whereas tryptophan was analyzed separately according to European Commission Regulation (EC) No 152/2009 III G [28]. For analysis of the fatty acids, seed samples were treated with trimethylsulphonium hydroxide (TMSH) to extant fatty acid methyl esters (FAMEs). FAMEs were analyzed using capillary gas chromatography and flame ionization detection (GC-FID) [29,30]. To analyze the glucosinolate concentrations, homogenized seed material was extracted and desulfated following the method described in DIN EN ISO 9167-1: 2013-12 [31]. The identity of individual desulfoglucosinolates was determined by high-performance liquid chromatography/electrospray-ionisation quadrupole time-of-flight tandem mass spectrometry (HPLC/ESI-QTOFMS) in positive ion mode. For this purpose, an Infinity 1290 Series UHPLC system interfaced via a dual Agilent jet stream electrospray ion source to an iFunnel Q-TOF mass spectrometer (G6550A, Agilent Technologies, Santa Clara, CA, USA) was used. For further instrument settings see Böttcher et al. [32].

Table 2. Harvest dates of OSR depended on the development stage of plants. Harvest 1 and harvest 2 of OSR plants grown under ambient soil temperature were performed about two weeks later than under elevated soil temperature. OSR was sown on 7 September 2016. The development stage is expressed as BBCH decimal code (DC) [22].

Harvests	Development Stage	Harvest Date Ambient Soil Temperature	Harvest Date Elevated Soil Temperature
Harvest 1	DC 31, stem elongation	27 March 2017	15 March 2017
Harvest 2	DC 65, full flowering	24 April 2017	10 April 2017
Harvest 3	DC 99, maturity	11 July 2017	11 July 2017

2.4. Statistical Analyses

Treatment effects on canopy height, aboveground biomass, specific leaf area, seed yield and seed quality of OSR were analyzed with linear mixed-effects models fitted by maximum likelihood (lme function of the R 3.4.2 nlme package). The model was as follows:

$$y_{hijklmn} = \mu + b_h + m_{hi} + p_{hij} + \tau_k + o_l + \vartheta_{ml} + \theta_{nl} + (\tau o)_{kl} + (\tau\vartheta)_{kml} + (\tau\theta)_{knl} + (\vartheta\theta)_{nml} + (\tau\vartheta\theta)_{kmnl} + e_{hijklmn}$$

where b_h, m_{hi} and p_{hij} are the random effect of the h^{th} block, i^{th} mainplot within h^{th} block and the j^{th} plot nested within the hi^{th} mainplot, respectively. τ_k, o_l, ϑ_{ml}, and θ_{nl} are the main effects of the k^{th} temperature, l^{th} roof, m^{th} precipitation frequency and n^{th} precipitation amount, respectively. Note that the latter two effects were applied under a roof only, thus the main effects are confounded with the corresponding interaction effects with roof and only one of both terms can be estimated. To account for this, we included arbitrarily the main effects into the model, but added the index l for all these effects. Interaction effects were denoted by parenthesis around the corresponding main effects. $e_{hijklmn}$ is the subplot error of observation $y_{hijklmn}$. It was assumed, that all random effects (block, mainplot, plot and subplot error) were independent and identically distributed with homogeneous variances σ_b^2, σ_m^2, σ_p^2, and σ_e^2, respectively. For the biomass of flowers we assumed a variance proportional to the number of flowers using the inverse of this number as weight. Residuals were graphically checked for homogeneous error variance and normal distribution using residuals-versus-fitted value plots and QQ (quantil-quantil) plots [33]. In case of extreme residuals, the corresponding data points were checked for plausibility. In total, two outliers were eliminated due to a lack of plausibility. After finding significant differences via global F test at $\alpha = 0.05$, Fishers least significant difference (LSD) test was used to find differences between means (again with $\alpha = 0.05$) and was performed with R package "agricolae". Ratios of means were calculated for presentation purpose only. Non-significant effects and differences with p-values between 0.05 and 0.1 were denoted as trend or tendency.

3. Results

3.1. Environmental Conditions and Biomass Harvests

Soil temperature increased by on average 1.7 ± 0.4 °C and 2.2 ± 0.4 °C in roofed and roof-control plots, respectively, during the entire growing period (7 September 2016 to 11 July 2017; Table 2). Precipitation patterns were manipulated from the beginning of summer to maturity (6 June 2017 to 11 July 2017). During that time period, the number of rainy days was ten days in control subplots. Consequently, in subplots where the precipitation frequency was reduced by 50%, the number of rainy days was five days. The ambient precipitation amount was 106 mm (control) in comparison to 79.8 mm in the reduced treatment (reduction of precipitation amount by 25%). Soil moisture in the 0–15 cm depth was not affected due to the water shortage or soil warming in comparison to control treatments.

3.2. Crop Development

Canopy height increased due to soil warming from the end of March 2017 (21 March 2017) to the beginning of May 2017 (2 May 2017) (Figure 2, Figure 3). Thus, the soil warming effect started when the inflorescence of OSR emerged (DC 50) and lasted until full flowering, at which time 50% of the flowers on the main racemes were open (DC 65). During this time period, the largest differences in canopy height were observed in April 2017: plants in the soil warming treatment were up to 40 cm higher than under ambient soil temperature. After flowering, plants under ambient soil temperature approximated the height of plants under elevated soil temperature.

Crop development of OSR was at first accelerated due to soil warming at the end of October 2016, when plants were quite young. Thus, plants under elevated soil temperature entered the development stage DC 15 (five leaves unfolded) seven days earlier than plants under ambient soil temperature (p = 0.05). Later, soil warming accelerated OSR development during spring: the stages indicating the end of flowering (DC 69, p = 0.017) and the beginning of fruit development (DC 71, p = 0.013) were entered seven and five days earlier than under ambient soil temperature. However, these differences in crop development between plants under ambient and elevated soil temperature disappeared by maturity (DC 99) and the final harvest (harvest 3) of all treatments took place on the same day (Table 2).

When the precipitation manipulation was started on 6 June 2017, crop development was between the stages of the middle of fruit development (DC 76) and beginning of ripening (DC 80). Overall, changes in precipitation patterns did not alter the phenology of the crop in all treatments. However, plants which grew under reduced precipitation amount and frequency tended to enter the end of the flowering stage (DC 69) on average one day after plants under ambient precipitation amount and frequency ($p = 0.069$).

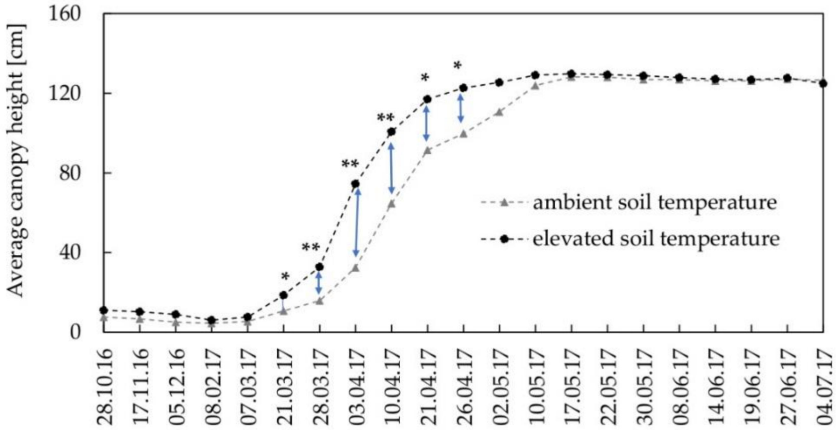

Figure 2. Canopy height measured between 28 October 2016 and 4 July 2017 at ambient and elevated soil temperature. Asterisks indicate significant differences (** $p \leq 0.01$, * $p \leq 0.05$) between plants grown under ambient and elevated soil temperatures; $n = 4$. Differences in average canopy height between treatments are labeled with blue arrows.

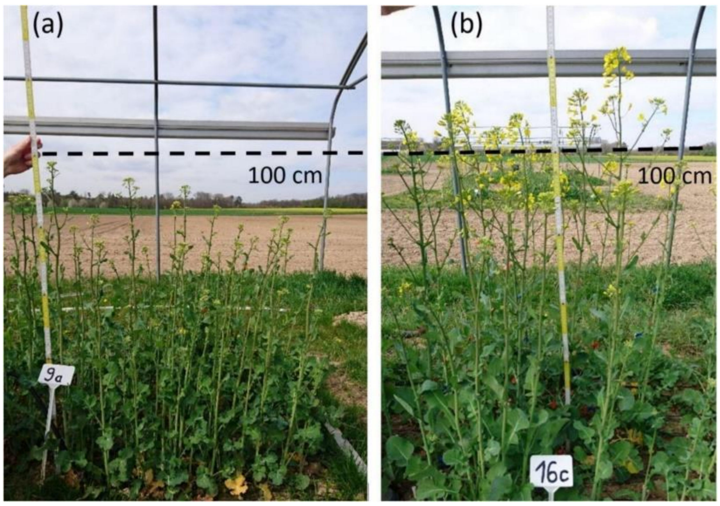

Figure 3. Differences in canopy height and crop development of OSR (oilseed rape) on 11 April 2017. Plants were grown either (**a**) under ambient soil temperature or (**b**) under soil warming. In (**a**) under control conditions, canopy height was up to 100 cm and plants were in development stage DC 59–DC 60. OSR under soil warming (**b**) was up to 120 cm high and in development stage DC 60–DC 65. The BBCH decimal code (DC) is used to define the development stages of the plants [22].

3.3. Biomass Allocation and Seed Yield

At stem elongation, aboveground biomass of OSR was not affected due to soil warming or changes in precipitation patterns (Figure 4). In contrast, soil warming tended to increase biomass of flowers by 138% ($p = 0.058$). In addition, a reduction in precipitation frequency increased biomass of flowers by 23% under ambient soil temperature, whereas the opposite was observed under elevated soil temperature (−19%) (Supplementary Materials Table S2). When precipitation amount was reduced, biomass of senescent leaves tended to increase by 60% ($p = 0.076$). SLA decreased by 11% if precipitation frequency was decreased under ambient soil temperature (Figure 5). In contrast, a reduction in precipitation frequency increased SLA by 14% under elevated soil temperature.

At flowering, SLA decreased by 5% under reduced precipitation frequency (Figure 5). Moreover, SLA increased by 18% due to a reduction in precipitation amount under ambient soil temperature. A decrease in SLA was observed under reduced precipitation amount in combination with elevated soil temperature (−17%). Roofing decreased SLA by 5% (data not shown).

At maturity, aboveground biomass, straw, and seed yield did not differ between treatments (Figure 4). Nevertheless, TSW increased by 7% under soil warming (Supplementary Materials Table S2). HI was increased under roofs at ambient (7%) and at elevated soil temperature (45%) as compared to non-roof-control plots (data not shown).

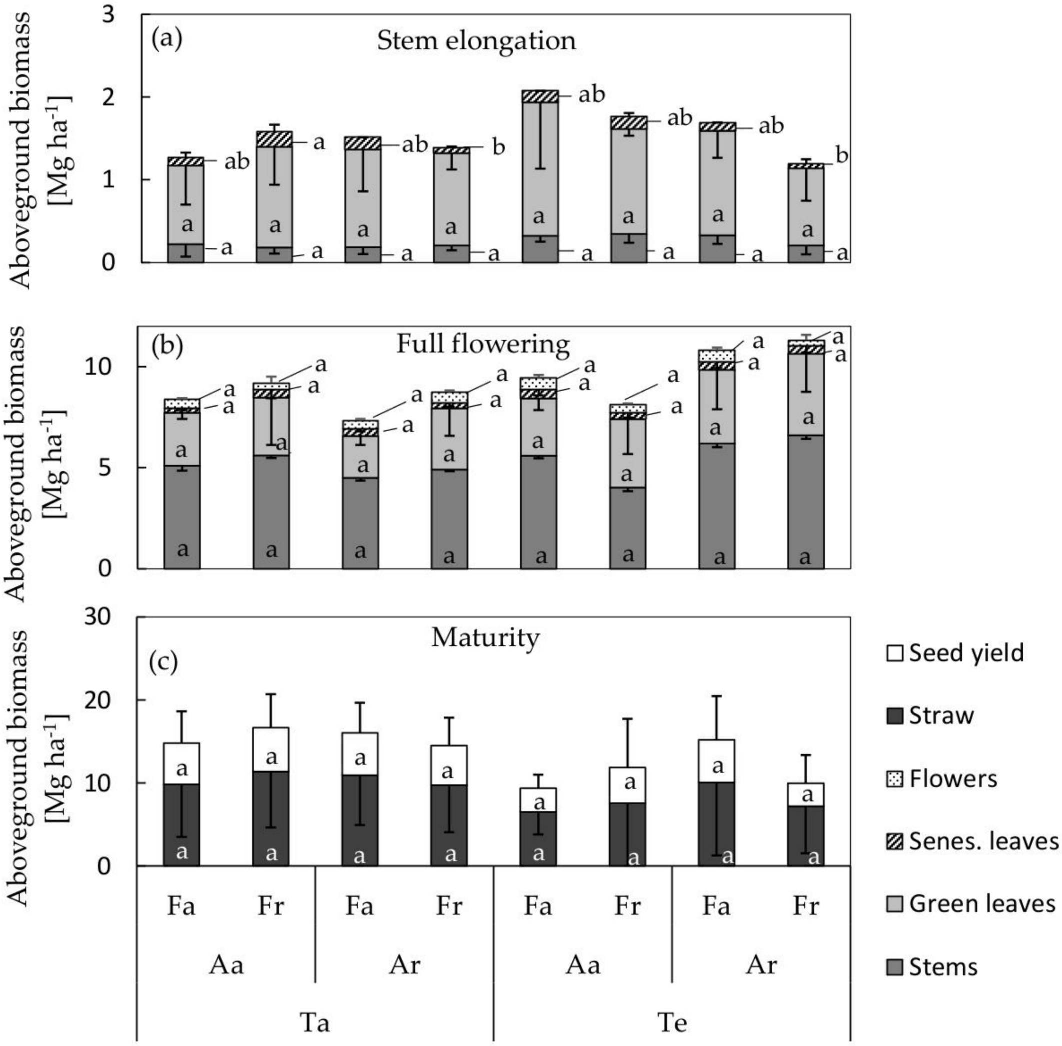

Figure 4. Aboveground biomass of OSR grown under ambient (Ta) or elevated (Te) soil temperature in combination with the following precipitation patterns: ambient (Aa) or reduced (Ar) precipitation amount and ambient (Fa) or reduced (Fr) precipitation frequency. Three harvests were performed: harvest 1 at stem elongation (DC 31) (**a**), harvest 2 at full flowering (DC 65) (**b**), and harvest 3 at maturity (DC 99) (**c**). The aboveground biomass fractions are shown as dry weight. Data are means ± standard deviations across four replicates (n = 4). Means with the same letter indicate a non-significant difference between these means based on Fishers least significant difference (LSD) test. Straw contains stem and senescent leave biomass. The data originate from OSR covered by roofs. The roofs had no effects on aboveground biomass of the three harvest dates. Abbreviation: Mg = megagram; ha = hectare; senes. = senescent.

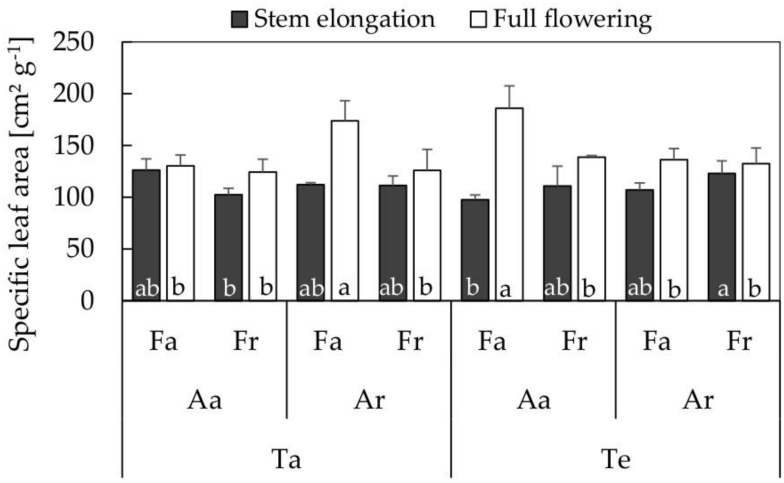

Figure 5. Specific leaf area (SLA) of OSR measured at stem elongation and full flowering. Data are means ± standard deviations across four replicates (n = 4). Means with the same letter indicate a non-significant difference between these means based on Fishers LSD test. The data originate from OSR covered by roofs. Effects of the roofing are mentioned in the text and therefore data of OSR planted under roofs are compared with OSR planted without roofs.

3.4. Seed Quality

The total protein content in seeds of OSR, as well as the concentrations of total amino acids on a per protein basis, remained unaffected under soil warming or changes in precipitation patterns (Supplementary Materials Table S3). Only the individual concentrations of essential and semi-essential amino acids [% protein] changed slightly under climate change conditions (Table 3): under reduced precipitation amount phenylalanine decreased (−1%), while isoleucine increased by 1% in response to reduced precipitation frequency. Additionally, interaction effects of the climate factors were observed: less precipitation amount at ambient soil temperature increased lysine by 1%. In contrast, lower precipitation amount at elevated soil temperature decreased concentration of lysine (−1%). Individual amino acids essential for children and semi-essential based on protein did not vary under the simulated climatic changes (Supplementary Materials Table S4). Roof effects on amino acid concentration were limited to a reduction of aspartic acid under ambient (−4%) and elevated (−1%) soil temperature (data not shown). The total and individual concentration of amino acids per unit dry weight did not vary between treatments (Supplementary Materials Tables S5 and S6).

The total oil content in mature seeds, as well as the total concentration of fatty acids on a per oil basis, did not vary between all treatments (Supplementary Materials Table S7). Correspondingly, the composition of fatty acids on a per oil basis remained more or less unaffected. However, the concentration of some saturated fatty acids changed in seeds of OSR (Table 3): the concentration of capric acid increased by 40% under ambient soil temperature and reduced precipitation amount. In contrast, 26% less capric acid was produced under elevated soil temperature and reduced precipitation amount. Moreover, reduced precipitation frequency decreased the lignoceric acid concentration at ambient precipitation amount (−3%) and increased it at reduced precipitation amount (+23%). Similar effects were found for saturated fatty acids on a per dry weight basis (Supplementary Materials Table S8). The concentrations of unsaturated fatty acids, e.g., of oleic acid, linoleic acid and linolenic acid per oil or dry weight basis, remained unaffected in all treatments (Supplementary Materials Table S9). Roofing affected the concentration of essential fatty acids: linoleic acid and linolenic acid decreased by 3% both under ambient and under elevated soil temperatures (data not shown).

Total glucosinolate concentration in OSR seeds was 10.9 ± 1.2 μmol g^{-1} DW (dry weight) in the control treatment (Supplementary Materials Table S10). Soil warming increased the total glucosinolate content by 26% to 13.8 ± 2.9 μmol g^{-1} DW in seeds. Looking at the concentration of individual glucosinolates, soil warming increased progoitrin, gluconapin and gluconapoleiferin by 29%, 24%, and 109%, respectively (Supplementary Materials Table S10). In contrast, a reduction in precipitation frequency decreased Gluconasturtiin by 14%. In roofed OSR, gluconasturtiin concentration was reduced by 9% and 38% under ambient and elevated soil temperatures (data not shown).

Table 3. Overall statistical analyses of impacts of soil warming (T), precipitation amount (A), precipitation frequency (F) as well as their interactions, on amino acid concentration (% protein) and fatty acid concentration (% oil) of mature OSR seeds.[1] Only parameters are presented that yielded significant effects of, or interactions between, treatments. [2]

Response Variable	T	A	F	T × A	T × F	A × F	T × A × F
Amino acid concentration [% protein]							
Essential [% protein]							
Isoleucine	ns	ns	**0.040**	**0.048**	ns	ns	ns
Phenylalanine	ns	**0.041**	ns	*0.086*	ns	ns	ns
Lysine	ns	ns	ns	**0.015**	ns	ns	**0.015**
Non-essential [% protein]							
Asparagine/aspartic acid	ns	ns	*0.077*	ns	ns	ns	ns
Glutamine/glutamic acid	*0.086*	ns	*0.070*	ns	ns	ns	ns
Proline	ns	*0.082*	ns	ns	ns	ns	ns
Alanine	ns	ns	ns	ns	*0.090*	ns	ns
Fatty acids concentration [% oil]							
Saturated fatty acids [% oil]							
Capric acid	ns	ns	ns	**0.023**	ns	ns	ns
Myristic acid	ns	ns	ns	ns	ns	ns	**0.047**
Lignoceric acid	ns	ns	ns	ns	ns	**0.048**	ns

[1] Data were tested by three-way analysis of variance (ANOVA) for main effects or interaction effects of the fixed factors T, A and F across four replicates ($n = 4$). Bold numbers indicate significant main or interaction effects of T, A, F ($p < 0.05$), numbers in italics indicate trend ($0.1 \geq p \geq 0.05$), ns = not significant ($p > 0.05$). Lipid numbers of the fatty acids: capric acid = C10:0; myristic acid = C14:0; lignoceric acid = C24:0. [2] Means and standard deviations of all determined amino and fatty acid concentrations are given in Supplementary Materials Tables S4 and S7.

4. Discussion

4.1. Crop Development and Crop Yield Parameters

Soil warming accelerated OSR development during early crop growth and during spring, which was similar to what we expected. It is known that in temperate climates an elevation in soil temperature can stimulate crop development [5], which has also been shown for winter wheat [4]. In the present study, OSR grown under elevated soil temperature was taller compared to ambient soil temperature from early growth stages to the beginning of May 2017 (DC 65). Afterwards, the soil warming effect on canopy height vanished, possibly due to a reduction in soil moisture due to higher air temperatures and less precipitation during summer compared to the period of spring. This is similar to an OSR field study in 2014, which was also performed within the HoCC experiment [34]. Accordingly, OSR was taller under soil warming until April and afterwards the huge difference in canopy height diminished until final harvest. Therefore, the effect of soil warming on canopy height appeared to decrease with increasing ambient temperatures in air and soil. In addition, soil warming resulted in a greater impact on smaller than on taller plants.

In our study, no change in total aboveground biomass was observed under soil warming at maturity, which was in agreement with a study using winter wheat [4]. In contrast, Bamminger et al. [34] found higher OSR aboveground biomass under soil warming at maturity. Increasing ambient air temperatures result in soil warming, which can alter plant–microbe interactions with impacts on the allocation of nutrients belowground in the rhizosphere [35]. It is possible that the higher mean air (+1 °C) and soil temperatures (+0.4 °C) in the study of Bamminger et al. [34] in 2014 compared to 2017 changed the nutrient availability for the plants and therefore promoted plant biomass production.

We observed stable seed yield under soil warming as well as under reduced precipitation amount and frequency. The achieved seed yield of OSR planted under control conditions (ambient soil temperature and precipitation) either under roofs or without roofs was 5.0 Mg ha^{-1} and 4.3 Mg ha^{-1}, respectively. Hence, seed yield of the control treatment correspond to the average winter OSR seed yield of 3.8 Mg ha^{-1} in the region Stuttgart in 2017. The average OSR seed yield in Stuttgart was achieved under normal agricultural practice [36].

To date, only a few studies investigating soil warming effects have been conducted on crop yield, e.g., of winter wheat [4,6] or maize [37] in temperate climates. However, elevated soil temperature can differ in their impacts on crop yield as compared to elevated air temperature. On the one hand, elevated air temperature can shorten the period of grain filling [38]. In low latitudes, elevation of air temperatures during the grain filling period are associated with a decrease in crop yield as a consequence of a reduction in plant photosynthesis, degradation of thylakoid components, and lower carbon exchange rate per unit of leaf area [39]. On the other hand, it has been observed that soil warming can affect the diversity and abundance of soil microorganisms [40]. Thus, alterations in plant–microbe interactions can occur due to impacts on the nutrient supply from the microbiome to the crop. Soil warming can stimulate the activity of soil microorganisms for a short time, but the microbial community seems to acclimatize to soil warming after a long exposure time [41]. This corresponds to the observations in the present study. Under permanent soil warming, we have observed stable seed yields of OSR. Thus, our results suggest an adaptation of soil microorganisms to permanent soil warming, assuming fewer alterations in plant–microbe interactions and no or minor impacts on the nutrition supply from the microbiome to the crop.

However, other explanations should also be considered. The detected stable OSR seed yield under soil warming in our study may have been due to sufficient water availability during the seed-filling period from beginning of April 2017 (DC 60) until end of June 2017 (DC 89), which mitigated evaporation consequences resulting from elevated soil temperature. This period included a wet April 2017 and high ambient precipitation amounts at the end of May 2017. Another explanation could be that soil warming by about 2 °C was too low to result in changes in factors such as the activity of soil microorganisms and the distribution of nutrients from the rhizosphere to the crop. In another soil warming experiment, crop yield of winter wheat remained unaffected at 5 °C elevated soil temperature to 100 mm depth [4].

In contrast, decreased OSR and cereal yields were observed in several studies with elevated air temperature [42–46]. Therefore, stable crop yields of OSR observed in our study under soil warming seemed to be a further indication that elevated temperatures in soil or air can result in different impacts on crop yield.

Besides elevated temperature, water scarcity also impacts seed yield. The time in plant life at which water scarcity appears is associated with effects on seed yield: reduced water amount during the periods of seed set or seed-filling can result in a decrease in seed yield [47]. Furthermore, Champolivier and Merrien [13] observed a reduction in seed yield in winter OSR after a period of water shortage which persisted from flowering until maturity. Our study did not detect reduced crop yields in OSR grown under reduced precipitation amount, presumably due to relatively wet conditions in the summer of 2017. Moreover, we hypothesized that seed yield of OSR decreases under reduced precipitation frequency. Accordingly, longer drought periods reduced the seed-filling period in OSR, as demonstrated by Hlavinka et al. [48] and Istandbulluoglu et al. [7]. In contrast, we found stable seed yield in OSR under reduced precipitation frequency and no change in seed-filling period, most likely due to high precipitation amounts during this period. Furthermore, our hypothesis that a simultaneous occurrence of soil warming, reduced precipitation amount, and reduced precipitation frequency have additive negative effects on seed yield of OSR could not be confirmed. This was perhaps due to moderate air temperatures below 30 °C from June to August 2017, resulting in moderate mean soil temperatures (24.6 °C) and sufficient water supply during the growing period. In addition, we observed relatively heavy rainfall events of between 20 and 30 mm at the beginning and end of June 2017. They appeared to mitigate negative effects of evapotranspiration and of a dry period in mid-June 2017 on soil water availability.

The SLA of crops is known to decline under elevated temperatures coupled with water shortage by a decrease in final leaf size [49]. Similarly, the SLA of OSR in the present study decreased under soil warming in combination with reduced precipitation amount.

Significant main effects and their interactions between amount and frequency of precipitation were detected in biomass allocation before the precipitation manipulation in 2017 began. Thus, biomass of flowers and senescent leaves increased, whereas SLA decreased, most likely as a result of plants producing smaller leaves under conditions of limited water availability. These are long-term effects of the precipitation manipulation, which is conducted every year during summer (always from June until August) in the same way at the same subplots since 2008. With regard to other long-term studies, which have been conducted mainly in grasslands, forests, and shrublands, variability in precipitation patterns over several years can lead to changes in, for example, soil respiration, as a consequence of alterations in soil structure or in the composition of the soil microbial community [50,51]. In our study, long-term changes in amount and frequency of precipitation may have resulted in an altered composition of the soil microbial community, which in turn affects availability of nutrients for the crops, and in the end, leaf size, leaf senescence, and biomass allocation of OSR.

4.2. Seed Quality

Alterations in seed yield, resulting from environmental stresses (e.g., temperature and precipitation patterns) can stimulate changes in the quantity of the seed oil produced [47]. Since seed yield did not vary under changes in soil temperature and precipitation in the present study, it appears that the total seed oil content remained unaffected in all treatments. By contrast with what is hypothesized, a reduction in precipitation amount neither increased the protein nor decreased the oil concentration, as had been found previously in OSR seeds when water scarcity was applied during the ripening period [13,14,52]. These contrasting results could be due to the fact that the reduced precipitation amount in this study was based on relatively high ambient precipitation amounts during the growing period of OSR. Thus, the simulated decrease in precipitation amount was too small to effect shifts in protein and oil concentrations.

In the present study, the observed changes in the essential amino acids phenylalanine, isoleucine, and lysine, when exposed to altered precipitation patterns alone or in combination with soil warming, seem to be negligible. In a previous study, an increase in those amino acids in leaves of OSR by 10% to more than 300% after two-day and four-day drought events was found [53].

The lipid biosynthesis of oil producing crops can be affected due to global warming, because an elevation in temperature can result in less desirable fatty acid profiles of vegetable oils [47]. Water availability is a second factor, one which can alter the composition of oilseeds since crops are prone to close their stomata under reduced water supply. This reduces carbon dioxide assimilation as well as sugar uptake by embryos [47]. Similarly, in the present study, minor changes in the fatty acid composition of OSR seeds were observed. Capric acid concentration decreased under elevated soil temperature combined with reduced precipitation amount and lignoceric acid increased if precipitation amount and precipitation frequency were reduced both. As far as we know, the function of saturated fatty acids in the metabolism of OSR is currently not fully understood. Capric acid, as a medium-chain fatty acid, is a valuable ingredient in OSR seed oil and used as feedstock in the production of biodiesel, cosmetics, lubricants, and surfactants [54]. Thus, a decrease in capric acid concentration could be unfavorable for the industrial usage of OSR seeds.

OSR is used for the production of edible oil or as a protein source for livestock so, for quality reasons, the concentrations of glucosinolates in mature seeds is restricted to 18 μmol g^{-1} [17], which was adhered to in all treatments. In this study soil warming increased glucosinolates in seeds. A positive correlation between increasing temperatures and glucosinolates was also shown in *Brassica oleracea* seeds [55]. Since glucosinolates are part of the plant defense reaction in the Brassicales order [56], water shortage during late growth stages can increase the glucosinolate concentration of OSR seeds at maturity [17]. In the present study, no effects of reduced amount and frequency of precipitation on the total glucosinolate concentration were observed. Several studies have reported that lower water availability will lead to a reduction in the number of seeds per plant, with glucosinolates distributed to fewer seeds, resulting in increased concentrations of glucosinolates in seeds [13,57]. In the present study, however, a stable seed yield was observed, which constitutes a stable sink capacity for glucosinolates. Most likely for that reason, total glucosinolate concentration did not change under water scarcity. A second explanation for why the precipitation treatments showed no effects on total glucosinolate concentration is that the growing period of OSR was relatively wet in 2017. Thus, the reduction in amount and frequency of precipitation was too mild to significantly increase the production of glucosinolates. Similarly, in a former field study with OSR grown in lysimeters, the glucosinolate concentration in seeds was not affected under mild drought stress conditions during the late developmental stage (pod filling stage) [17]. When determining the environmental effects on the quality of OSR grown across Victoria, Pritchard et al. [58] reported that the impact of cultivar on the glucosinolate content was greater than environmental impacts such as air temperature and rainfall amount.

5. Conclusions

In the present study, the interactive effects of soil warming and altered precipitation patterns on crop performance of winter oilseed rape were analyzed in a field experiment. At final harvest, crop development was similar in all treatments and no differences in aboveground biomass and seed yield were detected, suggesting that no adaptation to the date of sowing or harvest would be necessary under future climate change. Presumably, stable seed yield under soil warming was caused due to (1) an adaptation of soil microorganisms to permanent soil warming, due to (2) a sufficient water availability during the seed-filling period, which mitigated evaporation caused by elevated soil temperature, or (3) the soil warming by about 2 °C was too low to change the activity of the soil microorganisms and the distribution of nutrients from the rhizosphere to the crop. Furthermore, it is possible that seed yield did not change under reduced precipitation amount or frequency due to wet conditions during summer 2017. This underlines the need for long-term studies including a range of weather conditions during the vegetation period of oilseed rape. The mild environmental changes as simulated in the HoCC experiment, i.e., without pronounced periods of water shortage or drought, slightly changed the concentration of some amino acids, fatty acids, and glucosinolates. Therefore, irrigation of oilseed rape seems unnecessary to fulfil quality standards for seed marketing. It may be assumed that effects on seed yield and on the chemical composition of seeds will be more pronounced under a climate scenario with stronger increases in soil temperature and longer drought periods during summer months.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/4/520/s1: Table S1: Agricultural practices during the growing period, Table S2: Biomass of flowers at stem elongation, as well as thousand seed weight (TSW) and Harvest Index (HI) at maturity, Table S3: Total protein content (% dry weight), total amino acid concentration (% protein) and total concentration of amino acid types (% protein) in mature OSR seeds, Table S4: Individual amino acid concentration (% protein) in mature seeds, Table S5: Total amino acid concentration (% dry weight) and total concentration of amino acid types (% dry weight) in mature OSR seeds, Table S6: Individual amino acid concentration (% dry weight) in mature seeds, Table S7: Total oil content (% dry weight), total fatty acid concentration (% oil) and the concentration of individual saturated fatty acids (% oil) in mature OSR seeds, Table S8: Total and saturated fatty acid concentration (% dry weight) in mature OSR seeds, Table S9: Concentration of the unsaturated fatty acids: oleic acid, linoleic acid and linolenic acid (in % oil and in % dry weight) in mature OSR seeds, Table S10: Concentration of total and individual glucosinolates in mature OSR seeds.

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References

1. DaMatta, F.M.; Grandis, A.; Arenque, B.C.; Buckeridge, M.S. Impacts of climate changes on crop physiology and food quality. *Food Res. Int.* **2010**, *43*, 1814–1823. [CrossRef]
2. DWD. *Nationaler Klimareport. Klima—Gestern, Heute und in der Zukunft*, 3rd ed.; DWD: Offenbach am Main, Germany, 2017; pp. 1–46.
3. Zheng, D.; Hunt, E.R., Jr.; Running, S.W. A daily soil temperature model based on air temperature and precipitation for continental applications. *Clim. Res.* **1993**, *2*, 183–191. [CrossRef]
4. Patil, R.H.; Laegdsmand, M.; Olesen, J.E.; Porter, J.R. Growth and yield response of winter wheat to soil warming and rainfall patterns. *J. Agric. Sci.* **2010**, *148*, 553–566. [CrossRef]
5. Bowen, G.D. Soil temperature, root growth and plant function. In *Plant Roots: The Hidden Half*, 1st ed.; Waisel, Y., Eshel, A., Kafkaki, U., Eds.; Plenum Press: New York, NY, USA, 1991; pp. 309–330.
6. Gavito, M.E.; Curtis, P.S.; Mikkelsen, T.N.; Jakobsen, I. Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. *J. Exp. Bot.* **2001**, *52*, 1913–1923. [CrossRef]
7. Istanbulluoglu, A.; Arslan, B.; Gocmen, E.; Gezer, E.; Pasa, C. Effects of deficit irrigation regimes on the yield and growth of oilseed rape (*Brassica napus* L.). *Biosyst. Eng.* **2010**, *105*, 388–394. [CrossRef]
8. Bodner, G.; Nakhforoosh, A.; Kaul, H.-P. Management of crop water under drought: A review. *Agron. Sustain. Dev.* **2015**, *35*, 401–442. [CrossRef]
9. Barnabás, B.; Jäger, K.; Fehér, A. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* **2008**, *31*, 11–38. [CrossRef]
10. Walker, K.C.; Booth, E.J. Agricultural aspects of rape and other Brassica products. *Eur. J. Lipid Sci. Technol.* **2001**, *103*, 441–446. [CrossRef]
11. Weymann, W.; Böttcher, U.; Sieling, K.; Kage, H. Effects of weather conditions during different growth phases on yield formation of winter oilseed rape. *Field Crops Res.* **2015**, *173*, 41–48. [CrossRef]
12. Brown, J.K.M.; Beeby, R.; Penfield, S. Yield instability of winter oilseed rape modulated by early winter temperature. *Sci. Rep.* **2019**, *9*, 6953. [CrossRef]
13. Champolivier, L.; Merrien, A. Effects of water stress applied at different growth stages to *Brassica napus* L. var. oleifera on yield, yield components and seed quality. *Eur. J. Agron.* **1996**, *5*, 153–160. [CrossRef]
14. Bouchereau, A.; Clossais-Besnard, N.; Bensaoud, A.; Leport, L.; Renard, M. Water stress effects on rapeseed quality. *Eur. J. Agron.* **1996**, *5*, 19–30. [CrossRef]
15. Piper, E.L.; Boote, K.I. Temperature and cultivar effects on soybean seed oil and protein concentrations. *J. Am. Oil Chem. Soc.* **1999**, *76*, 1233–1241. [CrossRef]
16. Pipolo, A.E.; Sinclair, T.R.; Camara, G.M.S. Effects of temperature on oil and protein concentration in soybean seeds cultured in vitro. *Ann. Appl. Biol.* **2004**, *144*, 71–76. [CrossRef]
17. Jensen, C.R.; Mogensen, V.O.; Mortensen, G.; Fieldsend, J.K.; Milford, G.F.J.; Andersen, M.N.; Thage, J.H. Seed glucosinolate, oil and protein contents of field-grown rape (*Brassica napus* L.) affected by soil drying and evaporative demand. *Field Crops Res.* **1996**, *47*, 93–105. [CrossRef]
18. Alexander, J.; Auðunsson, G.A.; Benford, D.; Cockburn, A.; Cravedi, J.-P.; Dogliotti, E.; Di Domenico, A.; Férnandez-Cruz, M.L.; Fürst, P.; Fink-Gremmels, J.; et al. Glucosinolates as undesirable substances in animal feed.: Scientific Panel on Contaminants in the Food Chain. *EFSA J.* **2008**, 1–76.
19. Deutscher Wetterdienst. Vieljährige Mittelwerte: Tabelle B: Mittelwerte für den Bezugsstandort am Ende der Referenzperiode 1961–1990. Available online: https://www.dwd.de/DE/leistungen/klimadatendeutschland/vielj_mittelwerte.html (accessed on 3 February 2020).

20. Agricultural Technology Centre (LTZ) Augustenberg. Station Hohenheim. Available online: http://www.wetter-bw.de/ (accessed on 3 February 2020).
21. Poll, C.; Marhan, S.; Back, F.; Niklaus, P.A.; Kandeler, E. Field-scale manipulation of soil temperature and precipitation change soil CO_2 flux in a temperate agricultural ecosystem. *Agr. Ecosyst. Environ.* **2013**, *165*, 88–97. [CrossRef]
22. Meier, U. *Growth Stages of Mono- and Dicotyledonous Plants. BBCH Monograph*, 2nd ed.; Blackwell: Oxford, UK, 2001; pp. 29–34.
23. Umweltbundesamt. *Künftige Klimaänderungen in Deutschland—Regionale Projektionen für das 21. Jahrhundert*; Umweltbundesamt: Dessau, Germany, 2006; pp. 1–7.
24. Högy, P.; Poll, C.; Marhan, S.; Kandeler, E.; Fangmeier, A. Impacts of temperature increase and change in precipitation pattern on crop yield and yield quality of barley. *Food Chem.* **2013**, *136*, 1470–1477. [CrossRef]
25. European Commission. Regulation (EC) No 152/2009 III C. Determination of the content of crude protein. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* **2009**, *152*, 54/15–54/19.
26. European Commission. Regulation (EC) No 152/2009 III H. Determination of crude oils and fats. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* **2009**, *152*, 54/37–54/39.
27. European Commission. Regulation (EC) No 152/2009 III F. Determination of amino acids (except tryptophan). Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* **2009**, *152*, 54/23–54/32.
28. European Commission. Regulation (EC) No 152/2009 III G. Determination of Tryptophan. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* **2009**, *152*, 54/32–54/37.
29. DIN EN ISO 12966-1:2015-03. *Animal and Vegetable Fats and Oils—Gas Chromatography of Fatty Acid Methyl Esters—Part 1: Guidlines on Modern Gas Chromatography of Fatty Acid Methyl Esters (ISO 12966-1:2014)*; Beuth: Berlin, Germany, 2015.
30. Högy, P.; Franzaring, J.; Schwadorf, K.; Breuer, J.; Schütze, W.; Fangmeier, A. Effects of free-air CO_2 enrichment on energy traits and seed quality of oilseed rape. *Agr. Ecosyst. Environ.* **2010**, *139*, 239–244. [CrossRef]
31. DIN EN ISO 9167-1: 2013-12. *Rapssamen—Bestimmung des Glucosinolatgehaltes—Teil 1: HPLC-Verfahren*; Beuth: Berlin, Germany, 2013.
32. Böttcher, C.; Krähmer, A.; Stürtz, M.; Widder, S.; Schulz, H. Comprehensive metabolite profiling of onion bulbs (*Allium cepa*) using liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. *Metabolomics* **2017**, *13*, 10893. [CrossRef]
33. Kozak, M.; Piepho, H.-P. What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. *J. Agron. Crop Sci.* **2018**, *204*, 86–98. [CrossRef]
34. Bamminger, C.; Poll, C.; Sixt, C.; Högy, P.; Wüst, D.; Kandeler, E.; Marhan, S. Short-term response of soil microorganisms to biochar addition in a temperate agroecosystem under soil warming. *Agr. Ecosyst. Environ.* **2016**, *233*, 308–317. [CrossRef]
35. Singh, V.K.; Shukla, A.K.; Singh, A.K. Impact of climate change on plant-microbe interactions under agroecosystems. In *Climate Change and Agricultural Ecosystems: Current Challenges and Adaptation*; Choudhary, K.K., Kumar, A., Singh, A.K., Eds.; Woodhead Publishing: Duxford, UK; Cambridge, UK; Kidlington, UK, 2019.
36. Statistisches Landesamt Baden-Württemberg. Ernte der Hauptfeldfrüchte in Baden-Württemberg 2017: Artikel-Nr. 3354 17001, C II 1-j/17 (3). In *Statistische Berichte Baden-Württemberg*; Statistisches Landesamt Baden-Württemberg, Ed.; Statistisches Landesamt Baden-Württemberg: Stuttgart, Germany, 2017; pp. 1–7.
37. Stone, P.J.; Sorensen, I.B.; Jamieson, P.D. Effect of soil temperature on phenology, canopy development, biomass and yield of maize in a cool-temperate climate. *Field Crops Res.* **1999**, *63*, 169–178. [CrossRef]
38. Yadav, S.S.; Hegde, V.S.; Habibi, A.B.; Dia, M.; Verma, S. Climate change, agriculture and food security. In *Food Security and Climate Change*; Yadav, S.S., Redden, R.J., Hatfield, J.L., Ebert, A.W., Hunter, D., Eds.; John Wiley & Sons Ltd: Hoboken, NJ, USA, 2019.
39. Hatfield, J.L. Combined impacts of carbon, temperature, and drought to sustain food production. In *Food Security and Climate Change*; Yadav, S.S., Redden, R.J., Hatfield, J.L., Ebert, A.W., Hunter, D., Eds.; John Wiley & Sons Ltd: Hoboken, NJ, USA, 2019.

40. Aamir, M.; Rai, K.K.; Dubey, M.K.; Zehra, A.; Tripathi, Y.N.; Divyanshu, K.; Samal, S.; Upadhyay, R.S. Impact of climate change on soil carbon exchange, ecosystem dynamics, and plant-microbe interactions. In *Climate Change and Agricultural Ecosystems: Current Challenges and Adaptation*; Choudhary, K.K., Kumar, A., Singh, A.K., Eds.; Woodhead Publishing: Duxford, UK; Cambridge, UK; Kidlington, UK, 2019.
41. Kannojia, P.; Sharma, P.K.; Sharma, K. Climate change and soil. In *Climate Change and Agricultural Ecosystems: Current Challenges and Adaptation*; Choudhary, K.K., Kumar, A., Singh, A.K., Eds.; Woodhead Publishing: Duxford, UK; Cambridge, UK; Kidlington, UK, 2019.
42. Savin, R.; Stone, P.J.; Nicolas, M.E.; Wardlaw, I.F. Grain growth and malting quality of barley. 1. Effects of heat stress and moderately high temperature. *Aust. J. Agric. Res.* **1997**, *48*, 615–624. [CrossRef]
43. Faraji, A.; Latifi, N.; Soltani, A.; Rad, A.H.S. Seed yield and water use efficiency of canola (*Brassica napus* L.) as affected by high temperature stress and supplemental irrigation. *Agric. Water Manag.* **2009**, *96*, 132–140. [CrossRef]
44. Kutcher, H.R.; Warland, J.S.; Brandt, S.A. Temperature and precipitation effects on canola yields in Saskatchewan, Canada. *Agric. For. Meteorol.* **2010**, *150*, 161–165. [CrossRef]
45. Alemayehu, F.R.; Frenck, G.; van der Linden, L.; Mikkelsen, T.N.; Jørgensen, R.B. Can barley (*Hordeum vulgare* L. s.l.) adapt to fast climate changes?: A controlled selection experiment. *Genet. Resour. Crop Evol.* **2014**, *61*, 151–161. [CrossRef]
46. Ingvordsen, C.H.; Lyngkjær, M.F.; Peltonen-Sainio, P.; Mikkelsen, T.N.; Stockmarr, A.; Jørgensen, R.B. How a 10-day heatwave impacts barley grain yield when superimposed onto future levels of temperature and CO_2 as single and combined factors. *Agric. Ecosyst. Environ.* **2018**, *259*, 45–52. [CrossRef]
47. Singer, S.D.; Zou, J.; Weselake, R.J. Abiotic factors influence plant storage lipid accumulation and composition. *Plant Sci.* **2016**, *243*, 1–9. [CrossRef] [PubMed]
48. Hlavinka, P.; Trnka, M.; Semerádová, D.; Dubrovský, M.; Žalud, Z.; Možný, M. Effect of drought on yield variability of key crops in Czech Republic. *Agric. For. Meteorol.* **2009**, *149*, 431–442. [CrossRef]
49. Morison, J.I.L.; Lawlor, D.W. Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant Cell Environ.* **1999**, *22*, 659–682. [CrossRef]
50. Sowerby, A.; Emmet, B.A.; Tietema, A.; Beier, C. Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils. *Glob. Chang. Biol.* **2008**, *14*, 2388–2404. [CrossRef]
51. Beier, C.; Beierkuhnlein, C.; Wohlgemuth, T.; Penuelas, J.; Emmett, B.; Körner, C.; Boeck, H.d.; Hesselbjerg Christensen, J.; Leuzinger, S.; Janssens, I.A.; et al. Precipitation manipulation experiments-challenges and recommendations for the future. *Ecol. Lett.* **2012**, *15*, 899–911. [CrossRef]
52. Mailer, R.J.; Pratley, J.E. Field studies of moisture availability effects on glucosinolate and oil concentration in the seed of rape, (*Brassica napus* L.) and turnip rape (*Brassica rapa* L. var. silvestris (Lam.) Briggs). *Can. J. Plant Sci.* **1990**, *70*, 399–407. [CrossRef]
53. Good, A.G.; Zaplachinski, S.T. The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. *Physiol Plant* **1994**, *90*, 9–14. [CrossRef]
54. Dyer, J.M.; Stymne, S.; Green, A.G.; Carlsson, A.S. High-value oils from plants. *Plant J.* **2008**, *54*, 640–655. [CrossRef]
55. Del Carmen Martínez-Ballesta, M.; Moreno, D.A.; Carvajal, M. The physiological importance of glucosinolates on plant response to abiotic stress in Brassica. *Int. J. Mol. Sci.* **2013**, *14*, 11607–11625. [CrossRef] [PubMed]
56. Wittstock, U.; Burow, M. Glucosinolate breakdown in Arabidopsis: Mechanism, regulation and biological significance. *Arabidopsis Book* **2010**, *8*, 1–14. [CrossRef] [PubMed]
57. De March, G.; McGregor, D.I.; Séguin-Shwartz, G. Glucosinolate content of maturing pods and seeds of high and low glucosinolate summer rape. *Can. J. Plant Sci.* **1989**, *69*, 929–932. [CrossRef]
58. Pritchard, F.M.; Eagles, H.A.; Norton, R.M.; Salisbury, P.A.; Nicolas, M. Environmental effects on seed composition of Victorian canola. *Aust. J. Exp. Agric.* **2000**, *40*, 679–685. [CrossRef]