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# Cover crop residues mitigate impacts of water deficit on sunflower during vegetative growth with varietal differences, but not during seed development

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## ABSTRACT

Drought, as a major environmental factor that limits plant growth and photosynthesis, is a challenge for agriculture in the context of climate change. High temperatures and drought stress impact crops as a function of their stage of development and genotypic tolerance. Choosing adapted sunflower (*Helianthus annuus* L.) varieties and management practices can mitigate impacts of water deficit on growth, physiology and productivity, but with complex genotype × environment interactions. Cover crops (CC), used mainly as catch crops and/or green manure, can release mineral nitrogen after destruction, which influences growth and development of the following crop. Here, we studied how nitrogen released by CC residues can influence water deficit responses of sunflower. In semi-controlled experiments, using the high-throughput phenotyping platform Heliaphen, we tested impacts of water deficit on vegetative and post-flowering stages of four sunflower varieties in pots, in which CC residues of rye (*Secale cereale* L.) or vetch (*Vicia villosa* R.) had been incorporated before sowing. We studied impacts of water deficit during the vegetative stage on sunflower growth and transpiration and water deficit during the post-flowering stage on sunflower physiology and productivity. Under well-watered conditions, CC residues of vetch increased sunflower growth and productivity. Under water deficit conditions, CC residues mitigated the water-deficit response when applied during the vegetative stage, by limiting a decrease in growth, but they did not mitigate it post-flowering. Varieties responded differently to CC residues during vegetative and post-flowering stages. During seed development, severe water deficit cancelled out positive impacts of CC on productivity. Further research is needed to understand impacts of the intensity and period of water deficit on sunflower growth, physiology and yield following CC.

## 1. Introduction

Sunflower (*Helianthus annuus* L.) is known for its low water, nitrogen (N) and pesticide requirements and is thus the oilseed crop cultivated most in arid and semi-arid climates (irrigation or rainfed) and in temperate climates (primarily rainfed) (Garcia-Vila and Fereres, 2012). Sunflower cultivation is impacted by the increasing temperatures and drought stresses caused by climate change (Debaeke et al., 2017). In France, only 6% of total sunflower area is irrigated (Agreste, 2020), with a mean application of ca.70 mm that is usually split into two

applications (Agreste, 2017). Irrigation is significantly less common for sunflower than for other spring crops such as grain maize (*Zea mays* L.), 30% of whose area in France is irrigated with a mean of 150 mm, or soybeans (*Glycine max* (L.) Merr.), nearly 40% of whose area in France is irrigated with a mean of 140 mm (Agreste, 2017, 2020). Sunflower is also known for its low requirements for N fertilizer, with a mean application of 44 kg N/ha, compared to 144 kg N/ha for grain maize (Agreste, 2017). N fertilizers are usually applied at sowing and/or before the stage 14 leaves during vegetative growth (Lecomte, 2020).

Worldwide, sunflower is usually grown in 3–4 year rotations with

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cereals (e.g. common wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L. subsp. *durum*), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), soybean and beans (*Phaseolus vulgaris* L.). Sunflower is particularly well adapted to agroecological systems due to its short cycle, high disease and pest resistance and yield stability in low-input systems. In organic farming, mean sunflower yields can reach up to 90% of those in conventional farming in France (Lieven and Wagner, 2012). Sunflower maintains its productivity and profitability even when fertilizer and pesticide inputs are decreased in systems with cultivar mixtures, cover crops (CC) or sorghum, compared to a common 2-year durum wheat/sunflower cropping system with no decrease in chemical inputs (Bonnet et al., 2021).

The phenotypic plasticity (i.e. ability of a phenotype to adapt to environmental conditions) of sunflower is well-documented (Mangin et al., 2017; Nicotra et al., 2010; Temme et al., 2022). Sunflower is generally considered drought-tolerant, with larger ability to extract water than other crops (Anderson, 1979; Dardanelli et al., 1997; Garcia-Vila and Fereres, 2012). Water deficit changes plant water status when the soil water available does not meet the canopy's evaporative demand (Tardieu et al., 2018). Several studies have identified impacts of water deficit on leaf expansion, stomatal conductance and thus transpiration rate of sunflower genotypes (Connor et al., 1985; Connor and Jones, 1985; Connor and Sadras, 1992; Gimenez and Fereres, 1986). Before flowering, water deficit strongly influences leaf expansion, which influences growth, solar radiation interception and water use, while after flowering, stomatal conductance strongly influences the water status of a plant under water deficit, which influences cell extension and metabolic activity, and therefore photosynthesis, pollination and fecundation (Connor and Hall, 1997). Water deficit decreases leaf expansion and then stomatal conductance when the fraction of transpirable soil water (FTSW) falls below 0.85 and 0.40, respectively (Garcia-Vila and Fereres, 2012; Sadras et al., 1993). FTSW quantifies the degree of leaf expansion and transpiration responses to water deficit (Sinclair, 2005). Rate responses can be produced by different FTSW depending on the genotype (Mojayad and Planchon, 1994; Pereyra-Irujo et al., 2008a). Sunflower genotypes use two main drought-tolerance strategies: conservative or productive (Casadebaig et al., 2008; Duruflé et al., 2023; Rengel et al., 2012). In the conservative strategy, sunflower responds to drought by decreasing leaf expansion and closing stomata when the FTSW is relatively high, i.e. lower decrease of water availability. In the productive strategy, sunflower maintains its leaf expansion and transpiration rate despite the water deficit. Genotypes with the conservative strategy would be most adapted to environments with severe water deficits, while genotypes with the productive strategy would be most adapted to environments with short, frequent and moderate water deficits that alternate with well-watered periods.

Flowering and seed filling are the stages most sensitive to water deficit, which can decrease seed weight and number and oil content (Anderson, 1979; Andrianasolo et al., 2016, 2014). For example, water deficit can decrease pollination and fertilization during flowering and reduce photosynthesis, which provides most of the carbohydrates used for seed development and oil accumulation after flowering (Connor and Hall, 1997; Merrien, 1992). Water deficit after flowering can also increase leaf senescence and thus decrease yield by shortening the seed-filling stage (Moschen et al., 2019, 2017). However, higher N availability after flowering can decrease the senescence rate and increase yield, as observed in some studies (Goswami and Srivastava, 1988; Steer et al., 1985). After flowering, N supply helps maintaining the area of green leaves and photosynthesis, which influence the amount of solar radiation intercepted and carbon assimilated during seed filling, which in turn influence seed weight and oil content. Thus, water and N influence the growth, physiology and productivity of sunflower. Some studies have addressed effects of the interaction between water deficit and N supply on sunflower (Ahmad et al., 2014; Gonzalez-Dugo et al., 2010). N and water deficits are related because N mineralization depends on soil water and the solubilization of mineral N, which can be

taken up by roots, as simulated by the SUNFLO crop model (Quemada and Gabriel, 2016). Thus, water deficit can decrease the amount of mineral N in the soil available for crops. In addition, water deficit can influence plant physiological mechanisms such as leaf expansion, which can in turn influence crop N demand. Increasing the N supply can increase biomass production by increasing the amount of solar radiation intercepted and can increase yield under moderate water deficit (Nielsen and Halvorson, 1991). However, under severe water deficit, the larger leaf area and thus water use can increase water deficit and further decrease plant growth and yield (Ashraf et al., 2001; Nielsen and Halvorson, 1991).

Adoption of CC by farmers is an important tool in the design of agroecological cropping systems (Lamichhane and Alletto, 2022). CC, which are grown either at the same time as a cash crop or during the fallow period between two cash crops, provide multiple ecosystem services, such as decreasing nitrate leaching for most CC species, improving soil structure and fertility, protecting soils from erosion and regulating pests (Blanco-Canqui et al., 2015; Justes and Richard, 2017). Most CC are not harvested and are usually terminated before sowing the following crop by plowing them into the soil or leaving them on the soil as mulch (e.g. after crushing, chemical destruction or frost) (Justes et al., 2012; Justes and Richard, 2017). The duration of the fallow period can range from a few days (e.g. sowing a winter crop after a late harvest) to nearly 9 months (e.g. for a spring crop). Most of the ecosystem services that CC provide are related to the functionality, diversity and biomass that they produce, which depends greatly on how long they grow and the species and variety cultivated (Couëdel et al., 2018a; Thorup-Kristensen et al., 2003). Non-legume CC absorb large amounts of mineral N from the soil and decrease nitrate leaching effectively, while legume CC can fix atmospheric N through symbiosis and release it for the following cash crop (Couëdel et al., 2018b; Thorup-Kristensen et al., 2003). Legume CC release more N than non-legume CC do, as reflected in their lower C:N ratio (10–20), which also leads to faster mineralization of legume CC residues. The higher C:N ratio of non-legume CC (20–30) results in lower and slower mineralization of their residues. A moderate C:N ratio (> 25) can lead to N immobilization by micro-organisms that assimilate soil mineral N to meet their N requirements (Nicolardot et al., 2001). Thus, the CC chosen is a key factor that influences potential ecosystem services or disservices of CC and can influence the yield of the following cash crop. Effects of CC during the fallow period and their residues during the sunflower cycle have been described in studies that focused mainly on the final yield. In the literature, most legume CC (in a monocrop or mixture) increased or did not decrease sunflower yield (Ait Kaci Ahmed et al., 2022; Rosner et al., 2018; Wortman et al., 2012), while non-legume CC could decrease it (Adeux et al., 2021; Ait Kaci Ahmed et al., 2022; Meyer et al., 2022), perhaps due to N immobilization and water deficits caused by late destruction and spring drought. Drought during the sunflower cycle can influence the effects of CC (Adeux et al., 2021). However, little is known about how ecosystem services of CC (especially N mineralization of residues) can influence water-deficit responses of sunflower.

In the present study, we investigated morphological, phenological and physiological responses of sunflower varieties that differed in drought sensitivity using the semi-controlled environment of the Heliaphen phenotyping platform. At vegetative and post-flowering stages, we characterized sunflower plasticity among varieties in response to water deficit and CC residues, and how CC can mitigate negative impacts of water deficit on growth and physiological processes.

## 2. Materials and methods

### 2.1. Plant materials and growing conditions

The water-deficit experiments performed during the vegetative (code experiment 22HP05) and post-flowering (code experiment 22HP04) stages were performed in the outdoor Heliaphen phenotyping platform

at the INRAE research station, Auzeville-Tolosane, France (43° 31' N, 1° 29' E). The Heliaphen platform is a 650 m<sup>2</sup> outdoor phenotyping platform in which an autonomous moving robot weighs, irrigates and phenotypes plants grown in pots. The platform allows the environment (e.g. soil) and management factors (e.g. irrigation, fertilization) to be controlled and plant responses to water deficit to be measured precisely (Gosseau et al., 2019).

In each experiment, 144 plants, corresponding to 36 each of four sunflower varieties (MAS 86OL, MAS 89 M, MAS 98 K and CARRERA (MAS Seeds, France)), were grown in 15 L pots (28 cm diameter X 25 cm height). These varieties have contrasting water-deficit-response parameters for leaf expansion rate and transpiration rate, which were previously measured on the Heliaphen platform using the method of Casadebaig (2008) (Table 1). To represent different management strategies during the fallow period before sunflower cultivation, the pots contained either leached potting soil alone, to represent bare-soil fallow management, or the same soil supplemented with CC crushed rye (*Secale cereale* L.) (a grass) or CC hairy vetch (*Vicia villosa* Roth.) (a legume). All three treatments were performed under well-watered (WW) or controlled water deficit (WD) conditions that were automatically monitored using the robot. Under water deficit conditions, irrigation was stopped. Further details are presented in the description of experiments. Each treatment had six replicates.

Each pot was filled with 15 L of potting soil (PAM 2 Proveen, Bas Van Buuren, Netherlands), composed of black and blond peats, clay and substrate fertilizer (PG MIX 12–14–24), and covered with a 3 mm silicone sheet to prevent evaporation from the soil. Before the experiment began, the pots were irrigated abundantly for one week to leach mineral N from the soil in order to homogenize and decrease its N stock. Using a planter, two soil cores (0.3 L/core) were sampled from each pot at sowing, flowering (12 July) and harvest (29 August), and they were combined to represent the pot's soil. Contents of mineral N (i.e. nitrate and ammonium) in the soil were measured using a continuous flow auto-analyzer (Skylar 51,000, Skalar Analytic, Erkelenz, Germany) (standard NF ISO 14256–2). At sowing, mean mineral N content of the soil was 31 mg N.kg<sup>-1</sup>. On 12 July and 29 August, the mineral N content of the potting soil was low (< 15 mg N.kg<sup>-1</sup>) and had not been influenced by the CC treatment. On 29 Aug, the mineral N content of the soil was slightly higher under WD than under WW (Table 2).

The water status of plants was managed daily by the Heliaphen robot, as described by Gosseau et al. (2019). FTSW was used as an indicator of water deficit experienced by plants and estimated as follows:

$$FTSW = ATSW/TTSW \quad (1)$$

where ATSW is the available transpirable soil water, and TTSW is the total transpirable soil water.

ATSW and TTSW were calculated as follows:

$$ATSW = w_d - (w_{full} \times 0.39) \quad (2)$$

$$TTSW = w_{full} (1 - 0.39) \quad (3)$$

**Table 1**

Parameters for the water-deficit response in the SUNFLO crop model for the sunflower varieties studied (Casadebaig et al., 2008; Gosseau et al., 2019 and INRAE data). Values correspond to the model parameter describing the shape of the response of the physiological process to FTSW. The terms in parentheses indicate how early or late the process responds to water deficit (i.e. at lower or higher water deficit, respectively).

Variety	Leaf expansion rate	Transpiration rate
MAS 86 OL	-2.40 (mid-early)	-7.64 (mid-early)
MAS 89 M	-2.15 (early)	-13.98 (late)
CARRERA CLP	-4.55 (late)	-5.40 (early)
MAS 98 K	-3.68 (mid-late)	-8.68 (mid-late)

**Table 2**

Mean ( $\pm 1$  standard error) soil mineral nitrogen (mg N.kg<sup>-1</sup>) in the soil on 12 Jul (the end of flowering) (n = 4) and 29 Aug (harvest) (n = 12) by water regime (well-watered or water deficit) and cover crop treatment (vetch, rye or bare soil). n.d.: no data.

Regime	Treatment	12 Jul	29 Aug
Well-watered	Vetch	4.0 $\pm$ 0.5	7.0 $\pm$ 1.9
	Rye	6.0 $\pm$ 1.4	9.0 $\pm$ 4.6
	Bare soil	6.0 $\pm$ 1.4	9.0 $\pm$ 2.8
Water deficit	Vetch	n.d.	12.0 $\pm$ 4.9
	Rye	n.d.	15.0 $\pm$ 2.8
	Bare soil	n.d.	11.0 $\pm$ 4.8

where  $w_{full}$  is the mass of the pot at field capacity (g), and  $w_d$  is the mass of the pot on day  $d$  (g) (Blanchet et al., 2018).

CC were sown in Sep 2021 and sampled in Apr 2022 at INRAE, Auzeville-Tolosane. Fresh biomass including weeds (105 g fresh weight, corresponding to 24 or 30 g dry weight of hairy vetch or rye, respectively) were incorporated in the top 10 cm of soil in the pots five days before sowing sunflower on the Heliaphen platform. Rye and hairy vetch were chosen because they differ in the C:N ratio of their aboveground biomass. CC plots contained weeds (in majority, perennial ryegrass (*Lolium perenne* L.)) that were collected with the CC and whose aboveground biomass, N content and C:N ratio were measured before use (Table 3).

To characterize the CC residues, a sample of each was dried at 80°C for 48 h, weighed and ground to measure total carbon and N concentrations via elemental analyses based on the Dumas method (MicroVario Cube, Elementar, Langensfeld, Germany) (Hansen, 1989). Climatic metadata from CLIMATIK, the agrometeorological database of INRAE, were collected from the station's weather station (no. 31035002; 43° 31' 44.4" N, 1° 30' 14.4" E).

## 2.2. Vegetative and post-flowering water-deficit experiments

### 2.2.1. Vegetative water-deficit experiment

The vegetative water-deficit experiment (22HP05) was performed from 7 Apr 2022 (sowing) to 21 May 2022.

After sowing but before beginning the water deficit, plants were fertilized with 1.5 g of the oligo-element product Hortrilon® (1 g.L<sup>-1</sup>) (0.5% boron, 2.5% copper, 5.0% iron, 2.5% manganese, 0.5% molybdenum and 0.5% zinc), but no N fertilizer was applied. Under WD, irrigation was stopped from the B6-B7 stage (6–7 leaves) until 10 days later (i.e. 44 days after sowing). Meanwhile, under WW, plants were irrigated to maintain soil water at field capacity (FTSW = 1).

The plant transpiration rate (g.cm<sup>-2</sup>.d<sup>-1</sup>) was calculated as the amount of water lost per unit of total leaf area per day and then normalized by dividing the value under WD by the mean value under WW, corresponding to the ratio of the transpiration rate.

The total amount of water lost per plant (g.d<sup>-1</sup>) was estimated for each pot using an automated weighing robot (Blanchet et al., 2018; Gosseau et al., 2019).

Total leaf area (cm<sup>2</sup>) was predicted using a generalized linear model (GLM) based on features obtained from image analysis as described in Casadebaig et al. (2023). When weighing plants, a light-curtain sensor (Rapidoscan RS-C-025–768-ECT) was used to produce a black-and-white image of the plant's silhouette. Four features were then calculated by IPSO Phen software analysis of these images: projected area, hull area, bounding box area and plant height. The GLM was fitted to data from previous experiments 18HP10 and 19HP10 (available at <https://sunris-e-archive.toulouse.inra.fr>), using 1238 plant observations under both WW and WD. The GLM predicted total leaf area with a mean squared error of 2500 cm<sup>2</sup>. The relative error of 11% was sufficiently low to predict total plant area accurately in our experiments. The leaf expansion rate (cm<sup>2</sup>.d<sup>-1</sup>) was calculated as the difference between two



**Table 3**

Aboveground dry biomass (cover crop (CC), weeds and total (CC + weeds)) per pot (g), nitrogen (N) concentration (%) and C:N ratio of residues used in the study.

Residue	Biomass (g)			N concentration (%)			C:N ratio		
	CC	Weeds	Total	CC	Weeds	Total	CC	Weeds	Total
Hairy vetch	12.0	12.0	24.0	3.0	1.5	2.3	15.0	29.0	22.0
Rye	24.0	6.0	30.0	2.0	1.0	1.5	28.0	42.0	31.0

successive daily measurements and then normalized by dividing the value under WD by the mean value under WW, corresponding to the ratio of the leaf expansion rate.

### 2.2.2. Post-flowering water-deficit experiment

The post-flowering water-deficit experiment (22HP04) was performed from 7 Apr 2022 (sowing) to 29 Aug 2022 (harvest).

Under WD, irrigation was stopped from the flowering stage (F1) until FTSW in the soil in any pot decreased to 0.2. At that point, the pots were irrigated to maintain the FTSW at 0.2 until 5 Aug to impose a severe water deficit but ensure adequate seed filling for harvest. In contrast, under WW, plants were irrigated to maintain soil water at field capacity (FTSW = 1) until 5 Aug.

Plants were fertilized with 8.1 g of the oligo-element product Hortrilon® (4.6 g.L<sup>-1</sup>) over seven weeks (from 7 May to 1 Jul) and with 2.8 g of Peters® Professional 17–07–27 NPK (12 g L<sup>-1</sup>) once per week from 27 May to 1 Jul.

Plant height and collar diameter (cm) were measured, and the total number of leaves was counted at the flowering stage before the water deficit began. The flowering date of each plant was recorded.

Total leaf area was estimated at flowering using the method of Blanchet et al. (2018) because IPSO Phen software is calibrated only for sunflower plants at the vegetative stage.

The leaf senescence rate was determined as the slope of the leaf senescence ratio over time, which was calculated as the number of senescent leaves (i.e. with < 50% of green area) divided by the total number of leaves. The number of senescent leaves was counted at flowering and on 5, 13 and 29 Jul.

Chlorophyll, anthocyanin and flavonol contents of leaves and the N balance index (NBI) were measured on leaf N-5 with 3 readings per leaf, using an optical sensor (Dualex® Scientific +, FORCE-A, France), at flowering and on 30 Jun and 7, 13 and 21 Jul. Leaf stomatal conductance was also measured on leaf N-5 using a porometer (LI-600, LI-COR, Bad Homburg, Germany) on 30 Jun.

Aboveground biomass, seed weight, seed number and thousand-kernel weight (TKW) of each plant were measured at harvest (29 Aug). Aboveground biomass and seeds were ground to measure total N concentration via elemental analyses based on the Dumas method (MicroVario Cube). The harvest index was calculated as a plant's seed weight divided by its aboveground biomass at harvest. Water-use efficiency (g.L<sup>-1</sup>) was calculated as sunflower dry yield divided by rainfall plus irrigation from flowering until harvest. Nitrogen utilization efficiency (g seeds.g<sup>-1</sup> N) was calculated as seed weight divided by the amount of N in total aboveground biomass at harvest (Congreves et al., 2021; Keipp et al., 2019). Seed oil content was measured using a nuclear magnetic resonance analyzer (minispec MQ10, Bruker, Billerica, Massachusetts, USA). The amount of oil in seeds was calculated as plant's oil seed content multiplied by its seed weight.

### 2.3. Statistical analysis

All data were analyzed using R software (R Core Team, 2023). The linear model used consisted of three factors – CC treatment ( $\beta_1$ ), water regime ( $\beta_2$ ) and sunflower variety ( $\beta_3$ ) – and their interactions:

$$Y_{ijk} = \beta_0 + \beta_1 i + \beta_2 j + \beta_3 k + (\beta_1 \beta_2)_{ij} + (\beta_1 \beta_3)_{ik} + (\beta_2 \beta_3)_{jk} + (\beta_1 \beta_2 \beta_3)_{ijk} + \epsilon_{ijk}$$

Analysis of variance was used to test effects of treatments on plant

traits and productivity measured during each experiment. Temporal parameters (leaf expansion rate, transpiration rate) have been evaluated with the effect of time (as DAS) together with factors  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  in the above model and then tested for each day of measurement with this model. Post-hoc multiple comparisons (Tukey's test) were performed to compare treatments within a factor. Differences among treatments were considered significant at  $\alpha = 0.05$ .

## 3. Results

### 3.1. Impacts of cover crops and water deficit on seed production

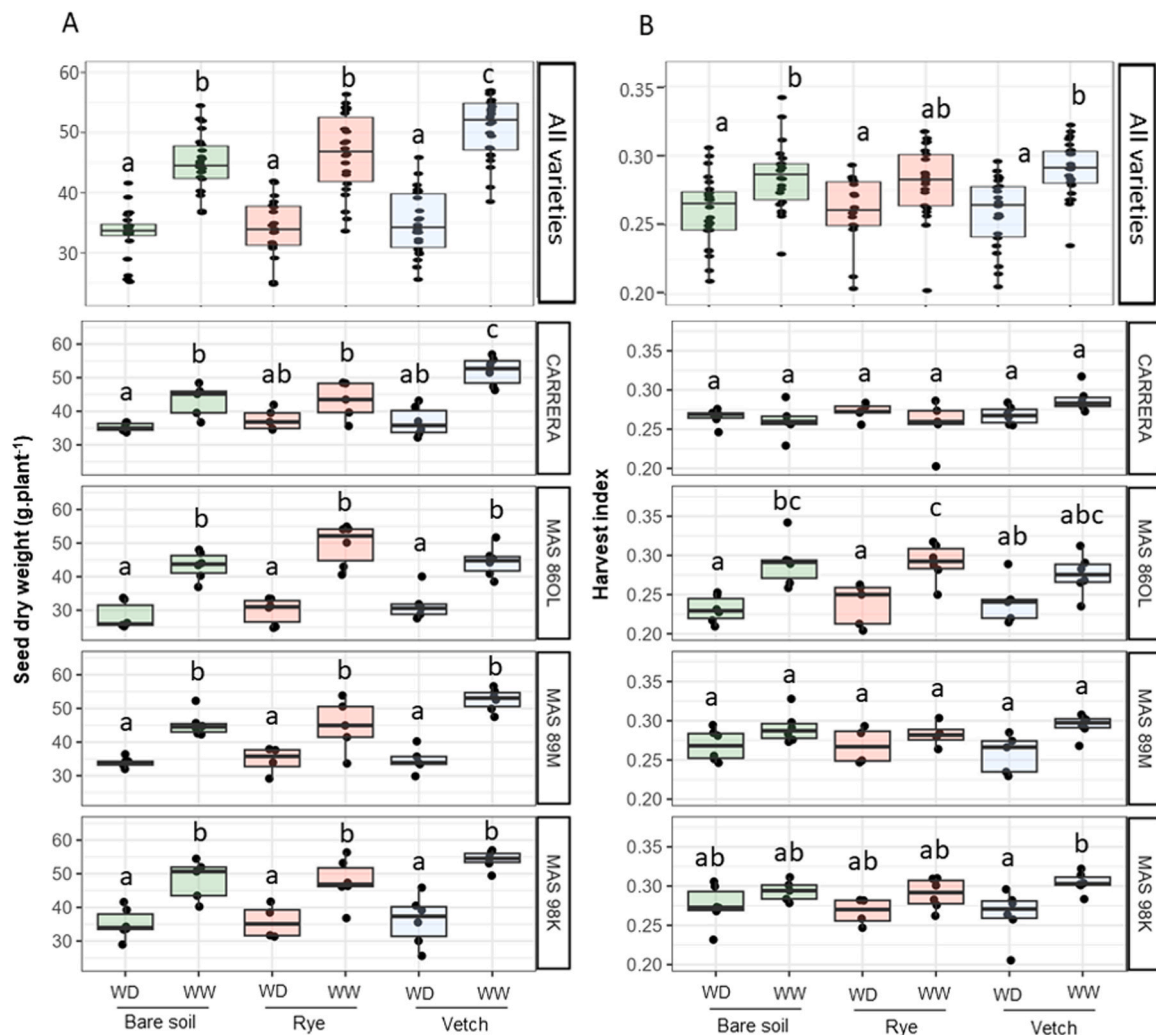
The experimental conditions in outdoor pots allowed effects of the interaction of CC and water deficit on yield to be studied. In the study, the mean seed weight (32 g.plant<sup>-1</sup>) is agreed with that estimated for the mean French sunflower yield in 2022 (31.5 g.plant<sup>-1</sup>, corresponding to the mean yield of 2.05 t.ha<sup>-1</sup> and assuming a stand of 65 000 plants.ha<sup>-1</sup>). For all four varieties, water deficit decreased seed weight, seed number and TKW by 28%, 19% and 11%, respectively, compared to those under WW ( $p < 0.001$ ). Seed number differed significantly among MAS 86OL, MAS 89 M, MAS 98 K and CARRERA ( $p < 0.001$ ) only under WD (787, 931, 940 and 1143 seeds respectively), as did seed weight (30, 34, 36 and 37 g.plant<sup>-1</sup> respectively) ( $p = 0.04$ ). However, CARRERA had the lowest TKW for both water regimes.

The presence of CC significantly increased seed weight, seed number and TKW averaged over varieties, compared to those with bare soil ( $p < 0.001$ ,  $p = 0.045$  and  $p < 0.001$ , respectively) but in a contrasting way depending on the type of CC and water regime (Fig. 1). Under WW, rye and vetch residues increased seed weight by 9% and 13%, respectively, and TKW by 8% and 6%, respectively, compared to those with bare soil ( $p = 0.01$  and  $p < 0.0001$ , respectively). Under WW, vetch residues increased seed number by 7%, compared to that with bare soil ( $p = 0.02$ ). Under WD, CC did not significantly influence seed weight, seed number or TKW. The interaction between CC and variety significantly influenced TKW ( $p = 0.04$ ), with the strongest effect for MAS 89 M under WW, with rye residues resulting in significantly higher TKW (48 g) than that with bare soil (38 g). In addition, vetch residues increased significantly seed weight compared to bare soil, only for CARRERA ( $p = 0.008$ ), particularly in WW conditions (Fig. 1).

The harvest index decreased by 9% under WD (Fig. 1). Harvest-index responses of varieties differed significantly between water regimes ( $p < 0.001$ ). Under WW, MAS 98 K had the highest harvest index (26.5), followed by MAS 86OL (24.8), MAS 89 M (24.7) and CARRERA (24.5), whereas under WD, MAS 86OL had a lower harvest index (15.5) than the other varieties did (mean of 18.6). Overall, CC did not influence the harvest index.

### 3.2. Impacts of cover crops and water deficit on seed quality

For all four varieties, water deficit generally had little effect on seed oil content, which was 52.9% under WW and 52.5% under WD ( $p = 0.04$ ) and decreased the amount of oil in seeds ( $p < 0.001$ ). However, varieties showed specific responses depending on the water regime, with significant differences among varieties under WW ( $p = 0.002$ ), in which seed oil content was highest for MAS 86OL (53.1%), intermediate for CARRERA (52.8%) and MAS 98 K (52.4%), and lowest for MAS 89 M (52.1%). Of note, vetch residues did not influence oil content but



**Fig. 1.** (A) Seed weight ( $\text{g. plant}^{-1}$ ) and (B) harvest index by sunflower variety as a function of water regime (water deficit (WD) or well-watered (WW)) and cover crop treatment (bare soil, rye or vetch). Each boxplot represents a mean of 6 replicates. Different letters indicate significant ( $p < 0.05$ ) differences among groups (water regimes by CC) for each variety according to Tukey's test.

increased (+12%) the amount of oil in seeds compared to those with bare soil, particularly under WW ( $p = 0.005$ ). This positive impact was related to the increase in seed weight in the presence of CC.

For all varieties, WD significantly increased seed N content by 32% compared to that under WW ( $p < 0.001$ ) and decreased the amount of N in seeds ( $p = 0.02$ ). Moreover, seed N content differed significantly among varieties under WD ( $p < 0.001$ ), being higher for MAS 86OL (2.4%) than for the other varieties (mean of 2.0%). CC did not influence seed N content, but vetch residues increased the amount of N in seeds by 16% compared to that with bare soil, particularly under WW ( $p = 0.02$ ). This increase was related to the increase in seed weight in the presence of vetch residues (Fig. 1 A).

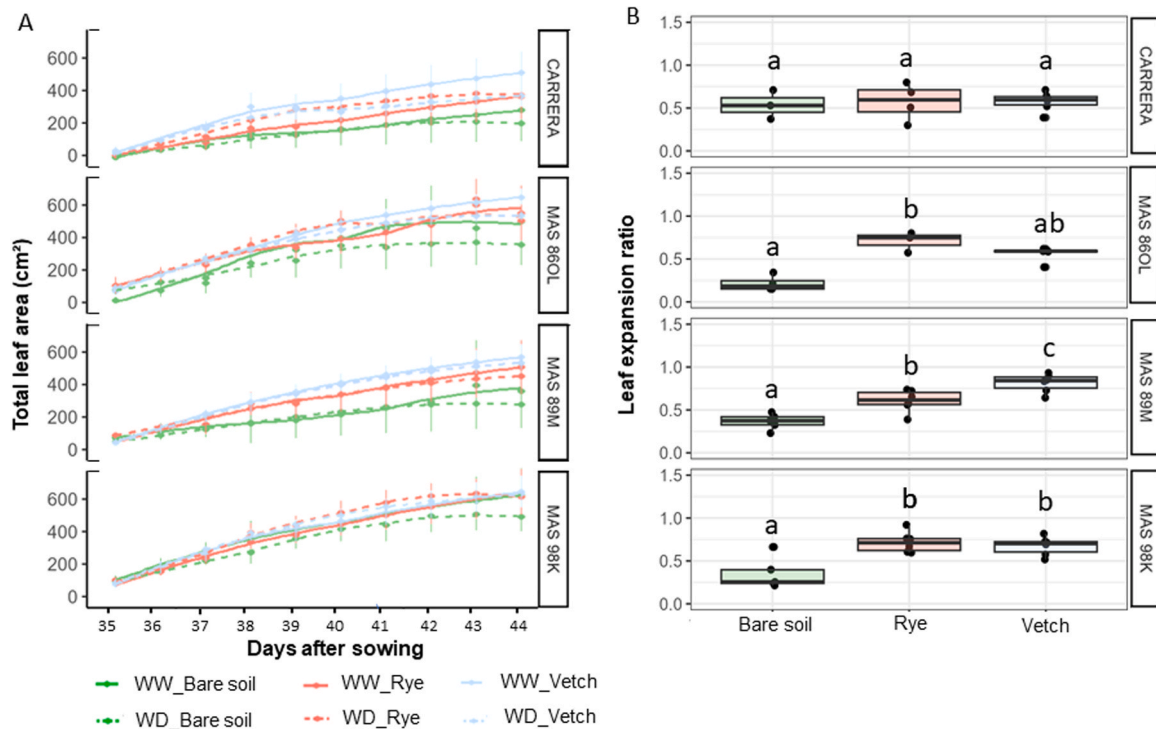
### 3.3. Cover crops increase early growth and mitigate impacts of water deficit during the vegetative stage

First, we tested a global model including time (DAS), CC, genotype, and water regime and identified that all factors impacted leaf expansion rate (respectively  $p_{\text{time}} < 0.001$ ,  $p_{\text{CC}} < 0.001$ ,  $p_{\text{genotype}} < 0.001$ ,  $p_{\text{water}} < 0.001$ ). In order to characterize when CC and water regimes acts, we studied their effects daily. Vetch and rye residues significantly increased the leaf expansion rate by 34% and 36%, respectively, compared to that of bare soil from 35 to 37 DAS, which corresponded to the B6-B7 stage under both water regimes ( $p < 0.001$ ). WD significantly decreased the

leaf expansion rate beginning 41 DAS compared to that under WW ( $p < 0.05$ ) (i.e. by 63% from 41 to 44 DAS, CC did not influence the leaf expansion rate under WW but increased it under WD compared to that with bare soil (i.e. by 80% for rye residues and by 260% for vetch residues,  $p < 0.0001$ ). The response of the ratio of the leaf expansion rate in the presence of CC differed among varieties ( $p = 0.001$ , Fig. 2). CC residues increased the ratio of leaf expansion rate of MAS 86OL (only in rye residues), MAS 89 M and MAS 98 K ( $p < 0.001$ ,  $p < 0.001$  and  $p = 0.001$ , respectively), by respectively 50% and 35% for MAS 86OL, 25% and 45% for MAS 89 M and 37% and 32% for MAS 98 K, illustrating that CC mitigated water stress (Fig. 2B). In contrast, CC did not influence the leaf expansion ratio of CARRERA.

At flowering, vetch residues significantly increased total leaf number by 3%, collar diameter by 4% and total leaf area by 11%, but not height, compared to bare soil, while rye residues had intermediate increases (Fig. 3). Importantly, CC did not influence the flowering date (Table A1). The morphological phenotypes of the four sunflower varieties at flowering studied did not respond differently to CC.

After flowering, water deficit increased the senescence rate of all varieties by 144% and decreased the biomass of their vegetative tissues at harvest by 20% ( $p < 0.001$ ). Overall, CC did not significantly influence the senescence rate (Fig. 3), but interestingly, an interaction among CC, water regime and variety did influence it ( $p = 0.05$ ). In the presence of vetch residues, compared to bare soil, MAS 89 M had the lowest



**Fig. 2.** Growth and water deficit response at the vegetative stage by sunflower variety as a function of water regime (water deficit (WD) or well-watered (WW)) and cover crop treatment (bare soil, rye or vetch). (A) Dynamics of total leaf area (from 35 days after sowing (DAS) (stage B6-B7) to 44 DAS). Means and standard errors represent 8 and 4 replicates under WD and WW, respectively. (B) Ratio of leaf expansion rate of sunflower under WD relative to that under WW from 7 to 10 days (41–44 DAS). Each boxplot represents a mean of 8 replicates under WD. Different letters indicate significant ( $p < 0.05$ ) differences among CC treatments by variety according to Tukey's test.

senescence rate (−19%) under WW and the highest senescence rate (+56%) under WD. Vetch residues increased the biomass of vegetative tissue at harvest ( $p < 0.0001$ ) by 10% under WW compared to that with bare soil, while rye residues yielded intermediate results (Fig. 3).

### 3.4. Impact of water deficit and cover crops on physiology

Similarly, to leaf expansion rate, we tested a global model including time (DAS), CC, genotype, and water regime and identified that all factors impacted transpiration rate (respectively  $p_{time} < 0.001$ ,  $p_{cc} < 0.001$ ,  $p_{genotype} < 0.001$ ,  $p_{water} < 0.001$ ). At the vegetative stage, WD decreased the transpiration rate beginning 41 DAS compared to that under WW ( $p < 0.001$ ), and the final transpiration rate decreased by 13% ( $p < 0.0001$ ). However, CC did not influence the responses of final transpiration rate under WD compared to those under WW, illustrating that CC did not mitigate effects of water deficit on the transpiration rate. Moreover, the ratio of the final transpiration rate differed among sunflower varieties only for MAS 89 M ( $p = 0.03$ ), which had a higher transpiration rate with vetch residues than rye residues (Table 4).

From flowering to harvest, water deficit increased water-use efficiency, with 4 and 1  $\text{g L}^{-1}$  of plants and seeds, respectively, under WW vs. 8 and 2  $\text{g L}^{-1}$  of plants and seeds, respectively, under WD ( $p < 0.001$ ). However, CC did not significantly influence water-use efficiency. In contrast, stomatal conductance confirmed an 82% decrease in transpiration on 30 June under WD ( $p < 0.001$ ). Stomatal conductance differed among varieties only under WW ( $p < 0.001$ ), being highest for CARRERA and MAS 89 M, intermediate for MAS 86OL and lowest for MAS 98 K. Interestingly, the interaction among CC, water regime, and variety had a significant effect ( $p = 0.003$ ): stomatal conductance of MAS 89 M was lower with rye residues under WD and higher with rye residues under WW.

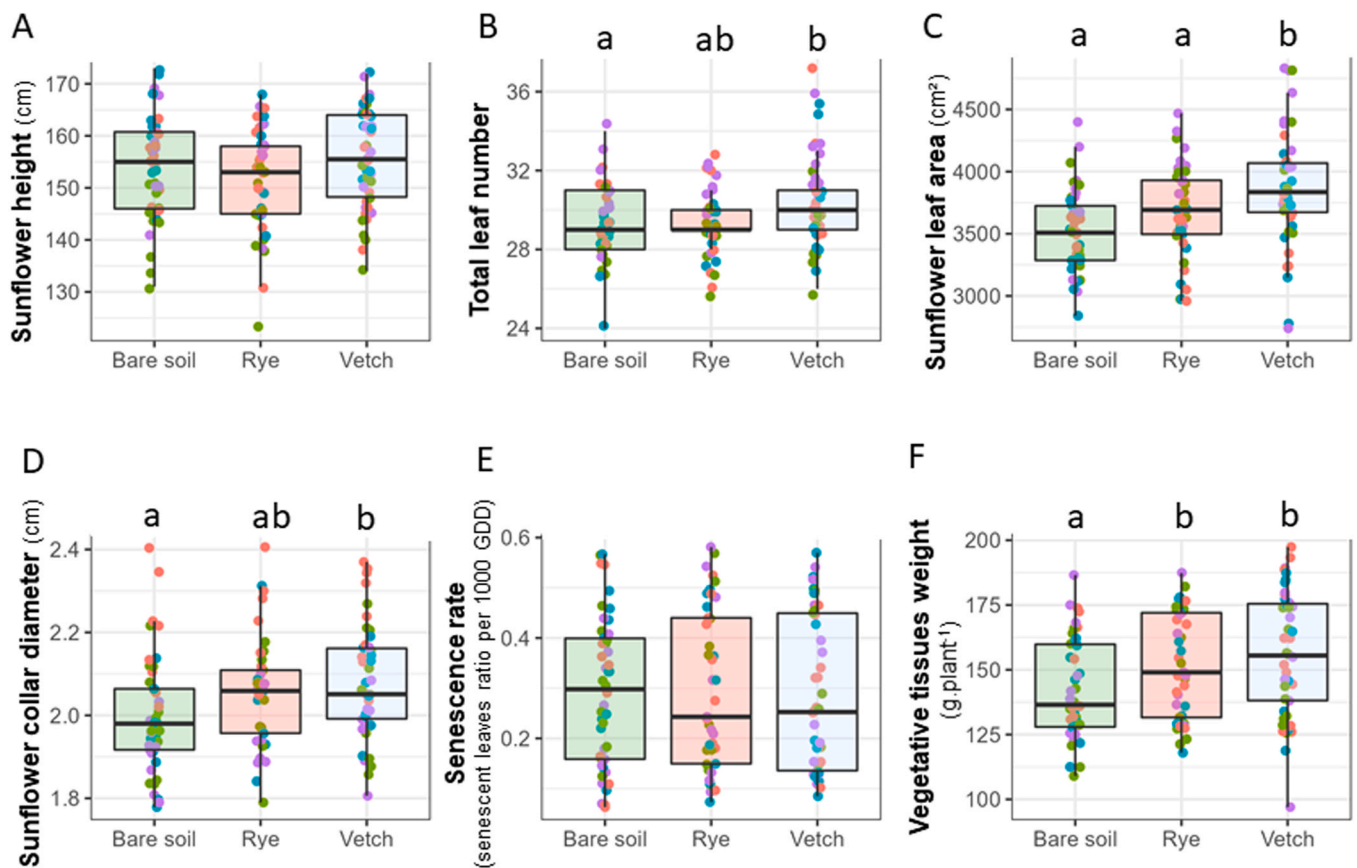
From 30 Jun to 21 Jul, the chlorophyll content of leaves and NBI decreased under WW by 28% and 21%, respectively, and increased

under WD by 61% and 221%, respectively ( $p < 0.001$ ). Water deficit significantly influenced the contents of chlorophyll (+135%), anthocyanin (+27%) and flavonol (−13%) of leaves and NBI (+177%) only on 102 DAS (21 Jul). Varieties differed significantly in chlorophyll and flavonol contents and NBI only under WD: MAS 89 M and MAS 86OL had the highest chlorophyll content and NBI and lowest flavonol content. CC did not significantly influence chlorophyll, anthocyanin or flavonol contents of leaves or the NBI at flowering and under WD (Table A1).

CC did not influence the N-utilization efficiency, N content of vegetative tissue or seed N content at harvest, but it did increase the amount of N in total aboveground biomass, particularly under WW ( $p = 0.006$ ). However, water deficit decreased N-utilization efficiency by 22%, increased N content of vegetative tissues by 8% and that of seeds by 32%, and decreased the amount of N in total aboveground biomass at harvest by 8%.

## 4. Discussion

Compared to bare soil, sowing CC before sunflower usually positively influences a variety of sunflower growth and development parameters, such as leaf area, leaf number, collar diameter, seed weight, seed number and TKW. These effects appeared mainly during the vegetative stage, although they also influenced sunflower throughout its cycle. The experiment performed during the vegetative stage showed effects of incorporating rye or vetch residues from the critical 6–7-leaf stage, which corresponds to the end of leaf differentiation and the beginning of floral initiation. During this stage, N supply can increase leaf surface area and its duration of photosynthesis, which strongly influences floral differentiation and thus potential seed production (Merrien, 1992; Steer et al., 1985). The type of the CC seemed to influence the magnitude of these effects: vetch usually had stronger effects than rye in the present study, and for most of the response variables studied. These results could



**Fig. 3.** Influence of cover crop treatment (bare soil, rye or vetch) on sunflower growth. (A) Height, (B) total leaf number, (C) total leaf area and (D) collar diameter at flowering; (E) Leaf senescence rate from flowering to harvest (leaf senescence ratio per 1000 growing degree days); (F) Vegetative tissues weight at harvest (g). Each boxplot represents a mean of 6 replicates. Different letters indicate significant ( $p < 0.05$ ) differences among CC treatments according to Tukey's test. Marker colors indicate varieties (CARRERA: red, MAS 86OL: green, MAS 89 M: blue, MAS 98 K: purple).

**Table 4**

Mean ratios ( $\pm 1$  standard error) of the leaf transpiration rate of water-deficit plants ( $n = 8$ ) relative to that of irrigated plants, 44 days after sowing, for each sunflower variety as a function of cover crop treatment: vetch, rye or bare soil. Different letters indicate significant ( $p < 0.05$ ) differences between treatments for each variety using Tukey's test.

Treatment	CARRERA	MAS 86OL	MAS 89 M	MAS 98 K
Vetch	0.83 $\pm$ 0.15 a	0.87 $\pm$ 0.06 a	0.95 $\pm$ 0.07 b	0.82 $\pm$ 0.03 a
Rye	0.74 $\pm$ 0.06 a	0.73 $\pm$ 0.05 a	0.70 $\pm$ 0.03 a	0.84 $\pm$ 0.05 a
Bare soil	0.85 $\pm$ 0.09 a	0.63 $\pm$ 0.16 a	0.78 $\pm$ 0.09 ab	0.84 $\pm$ 0.04 a

be due to legumes having higher N content than grasses and other botanical families used as CC (Couédel et al., 2018b; Tribouillois et al., 2016), leading to a lower C:N ratio and faster mineralization of a larger amount of N (Justes et al., 2009). Experimental and modelling studies of maize have shown that mineralization peaks of CC depend on the C:N ratio, which in turn depends on the stage at which CC are destroyed and returned to the soil, and that legumes generally release N earlier than non-legumes, with a time lag of 2–6 weeks depending on the CC (Alletto et al., 2022; Chim et al., 2022). This time lag may have been responsible for the differences in sunflower response observed in the present study. Furthermore, CC can have an impact on the soil mineral N content during their growth, through rhizodeposition and decomposition of litter material for legume crops and high N absorption for non-legume CC (Couédel et al., 2018a). Considering this other ecosystem function of CC might affect the magnitude of CC effect.

Positive effects of CC under WD were identified for the leaf expansion rate during the vegetative stage compared to that with bare soil. In

particular, vetch and rye residues mitigated the decrease in leaf expansion caused by water deficit by nearly 40% which can be explained by several processes. For example, N, which occurs in many plant cell components (e.g. chlorophyll, amino acids, nucleic acids), strongly influences cell metabolism, which in turn influences crop response to water deficit. One influential process could be an increase in N-containing osmolytes (e.g. glycine-betaine, amino acids) via higher N uptake, which decreases osmotic potential due to accumulation of solutes and thus maintains a higher turgor potential, which may help limit the decrease in leaf expansion, as shown by Hussain et al. (2016). This result is consistent with the biosynthesis of osmolytes under water deficit, which contributes to osmotic adjustment, as shown in our previous study under the same experimental conditions (Fernandez et al., 2019). Osmolytes could also be involved in other mechanisms associated with tolerance to water deficit, such as detoxification of reactive oxygen species (ROS) (Maury et al., 2011). Altogether, the increased N supply that CC provide can improve the water relations and photosynthetic activities of sunflower under water deficit, as observed in certain studies (Ashraf et al., 2001; Hussain et al., 2016).

The effects of CC on sunflower biomass production, explained mainly by the increase in leaf area in the presence of vetch residues, were observed until flowering, the stage with the largest leaf area before senescence begins. This positive and contrasting effect on leaf area index depending on the type of CC (legume or grass) agrees with observations in other studies (Thind et al., 2007). In the present study, CC did not influence the date that senescence began or the dynamics of senescence, regardless of the water regime. Keeping photosynthetic activity high by slowing senescence is a major challenge if the goal is to lengthen the seed-filling stage and redistribute nutrients efficiently (Aguirrezábal



et al., 2003; Moschen et al., 2017). Under normal growth, a leaf's senescence is governed by its developmental age, which depends on hormones and other regulatory factors. Leaves can also senesce prematurely under stressful environmental conditions (e.g. water or mineral deficit) (Großkinsky et al., 2018), but maintaining an optimal N supply during seed filling can delay this (Goswami and Srivastava, 1988). CC must therefore be able to generate effects based on combining species that decompose rapidly (to supply N at the beginning of the cycle) with those that decompose slowly (to supply N during seed filling), which highlights the importance of studying mixtures to identify species with complementary ecosystem functions (Lamichhane and Alletto, 2022).

Recent field experimental and modelling studies have shown that using legumes as CC can increase sunflower productivity compared to using bare soil or other botanical CC families (Adeux et al., 2021; Ait Kaci Ahmed et al., 2022; Hakyemez and Kavdir, 2008; Meyer et al., 2022). The present study shows that under non-limiting water conditions, CC, especially vetch, increased seed weight and number (i.e., by 13% and 7% for vetch, respectively) compared to those with bare soil. These increases can be explained by greater carbon assimilation via increased interception of solar radiation, mainly due to an increase in leaf area, although we observed no change in senescence rate, which can also influence sunflower productivity (Connor et al., 1985). We also observed no differences in harvest indexes and thus in the redistribution of nutrients to seeds between the treatments with and without CC, which agrees with observations of Escalante-Estrada et al. (2022), who observed no increase in the redistribution of nutrients to sunflower seeds when the amounts of N inputs increased. Nonetheless, since total sunflower biomass (i.e. stems, leaves and seeds) increased with CC, especially vetch under WW, the amount of N in seeds also increased compared to that with bare soil.

Under WD, however, CC had mixed effects on seed weight and number, with a decrease in seed number with all CC treatments, possibly due to an increase in abortion (Yegappan et al., 1982), and a general decrease in seed weight regardless of treatment. These results are consistent on one hand with those of other studies that highlighted major effects of high water deficit on sunflower productivity, even though it is one of the most water-tolerant summer crops (Debaeke et al., 2017; Harris et al., 1978), and on the other hand to results of Hunter et al. (2021) where legume cover crop did not result in maize yield increase in drought conditions. Some consequences of water deficit after flowering include a decrease in the harvest index (Ferreles et al., 1986; Soriano et al., 2002) and stomatal conductance (Andrianasolo et al., 2016; Sobrado and Turner, 1986), and an increase in the senescence rate (Großkinsky et al., 2018). Physiological reactions of plants to water deficit usually include production of ROS and, under certain conditions, complementary production of phenolic compounds known to trap these ROS (Ebrahimian and Bybordi, 2012; Ghobadi et al., 2013). The analysis of pigments in the present study did not identify effects of CC on the production of compounds that mitigate effects of water deficit. Further research should be performed on this subject. Furthermore, in our semi-controlled experiment, growing sunflower in 15 L pots without irrigation and in high temperature open-air can amplify the water deficit response compared to field conditions.

In the present study, CC did not influence the oil and N contents of the seeds, which is consistent with results of multi-year experiments on sunflower cultivated after bare soil or CC to an increase in leaf senescence or decrease in photosynthesis, as observed in other studies (Andrianasolo et al., 2014; Hall et al., 1995). Nonetheless, as mentioned, given equivalent seed N and oil contents, since seed weight and number increased after CC, the amount of N and oil in seeds also increased compared to those with bare soil. These results are consistent with those of previous studies that highlighted the importance of seed number in sunflower N responses (Ali and Ullah, 2012; Steer et al., 1984).

We also sought to better understand the varietal response of sunflower to water deficit by studying the interactions with the effects

generated by the introduction of CC. In response to water deficit, sunflower shows genotypic variability in growth and physiology (Andrianasolo et al., 2016; Casadebaig et al., 2008; Pankovic, 1999; Pereyra-Irujo et al., 2008b; Virgona et al., 1990) and in productivity. To study these responses, we chose four varieties with contrasting conservative or productive responses to water deficit, which result in differences in leaf expansion and transpiration rates (Andrianasolo et al., 2016; Casadebaig et al., 2008). As mentioned, interactions between the water regime and variety significantly influenced seed weight, seed number, TKW and harvest index, with larger differences among varieties under WD. Thus, under WD, seed weight was highest for CARRERA and MAS 98 K and lowest for MAS 86OL because CARRERA and MAS 98 K had the highest seed number. The harvest index also varied among the varieties and in interaction with the water regime. In particular, it decreased under WD and was related to a decrease in seed number, as observed for MAS 86OL and in other studies (Ferreles et al., 1986).

Under WD, the productivity of the varieties did not respond differently to the use of CC, but their growth, transpiration and senescence did. Only CARRERA did not mitigate the decrease in leaf expansion under WD, potentially due to genotypic variability in osmotic adjustment in response to water deficit (Maury et al., 2000). The experiments highlighted other genotypic differences; for example, only MAS 89 M reacted differently under WD in the presence of vetch, with a higher transpiration rate at the vegetative stage and higher senescence rate post-flowering, with no change in its productivity. Further research is required to better understand this physiological response, but MAS 89 M's strategy, which is to maintain transpiration under water deficit, may increase senescence because the variety produces more biomass at flowering in response to the N supplied by vetch residues. These results are consistent with those that illustrate negative effects of mineral N inputs under water deficit, because they increase transpiration (Ashraf et al., 2001; Nielsen and Halvorson, 1991). Thus, they could help farmers choose which CC to plant before sunflower depending on the practices used (e.g. irrigation or not) and the soil and climate conditions (e.g. water retention, rainfall), which result in different drought risks.

## 5. Conclusion

The study estimated impacts of CC and water deficit on the growth, physiology and productivity of sunflower. CC residues mitigated the response to water deficit at the vegetative stage by limiting the decrease in leaf expansion rate but did not do so at the post-flowering stage. CC residues induced the release of mineral N during the vegetative stage, which increased the leaf expansion rate and mitigated the decrease in leaf expansion under WD by 40% during the vegetative stage. This increase in growth caused by CC residues, particularly of vetch, can explain sunflower's higher productivity in the presence of CC, which could be due mainly to the increase in carbon assimilation, with more leaf area at flowering, via an increase in interception of solar radiation by leaves and in redistribution of assimilates from vegetative tissues to seeds. However, post-flowering water deficit canceled out the positive impacts of CC on productivity by decreasing carbon assimilation and redistribution of assimilates. Under water deficit, sunflower varieties responded differently in the presence of CC, specifically growth and the transpiration rate during the vegetative stage and the senescence rate during the post-flowering stage. Our results can guide variety selection and crop management to better match the CC residue N release with the response of subsequent cash crop during vegetative and post-flowering phase. Future experiments should focus on post-flowering water deficit responses of sunflower varieties and their productivity in the presence of CC under different intensities and periods of water deficit.

## CRedit authorship contribution statement

**Nicolas Blanchet:** Data curation, Investigation, Resources. **Pierre Casadebaig:** Formal analysis, Methodology, Software, Writing – review

& editing. **Nicolas Bernard Langlade**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Lucie Souques**: Conceptualization, Data curation, Formal analysis, Validation, Writing – original draft. **Lionel Alletto**: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

Data will be made available on request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eja.2024.127139](https://doi.org/10.1016/j.eja.2024.127139).

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