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Original article

# Contrasting effects of cover crops on earthworms: Results from field monitoring and laboratory experiments on growth, reproduction and food choice

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ABSTRACT

Cover crops are an essential element of sustainable agriculture and can affect earthworm populations. In a field trial, we investigated the effects of four cover crop treatments: radish (*Raphanus sativus* var. *longipinnatus* B.; at high and low seed density), black oat (*Avena strigosa* Schreb.) and Sudan grass (*Sorghum sudanese* M.) on earthworms under two irrigation regimes. The two parallel field trials (irrigated and rainfed) demonstrated the significance of soil moisture for earthworm abundance with lower numbers under rainfed black oat and Sudan grass compared with moister bare fallow in autumn ($P < 0.05$). Soil moisture content changed from autumn to spring and was highest under Sudan grass in both irrigation regimes ($P < 0.05$). Earthworm numbers equalised and were then similar in all treatments, but under rainfed cover crop treatments, earthworm populations gained 62.3 g $g^{-1}$ in biomass from autumn to the following spring ($P < 0.05$). Laboratory experiments showed the importance of N content and more palatability of low C:N ratio radish for growth rate of juvenile *Aporrectodea longa* and cocoon production by *Aporrectodea caliginosa*. These two earthworm species showed a different preference in choice chamber experiments between roots and shoots. Radish was consumed first in three out of four experiments. Field and laboratory experiments highlighted the effects of cover crops on earthworm abundance, reproduction and development. Overall, our results showed that cover crops can support earthworm development, but under field conditions, soil moisture is more important. In the short-term, this can lead to a trade-off between plant biomass production and earthworm numbers.

## 1. Introduction

Cover crops are an essential component in crop rotations to protect soil from erosion and support soil biota such as earthworms [1–4]. Earthworms are known for their beneficial effects on soil structure, the provision of plant-available nutrients and their ability to reduce plant pathogens [3,5–8]. Therefore, increasing earthworm abundance by provision of cover crops can lead to enhanced agricultural sustainability [3,4,9–11].

Due to biomass production, cover crops provide ecosystem services for subsequent cash crops, such as nutrient cycling, suppression of weeds, prevention of water runoff, water losses due to shading of the soil surface from evaporation and therefore reduction of soil temperature [1,11–15]. However, a high biomass production of cover crops can also decrease soil moisture due to water uptake in the growing season and a low soil temperature in spring can retard cash crop emergence and be beneficial for soil-borne pathogens [13,16]. Nevertheless, for earthworm populations, an adequate soil moisture is paramount, therefore a trade-off between plant biomass production and earthworm abundance is possible, especially under dry conditions [10,13,17–19].

In an agricultural setting, earthworm abundance is increased by adequate soil moisture, food supply and a reduced soil tillage [20–24]. A

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low C:N ratio, as present in Fabaceae (e.g. *Pisum sativum* L.) is considered favourable for earthworms, whereas the amount of available plant biomass itself seems less important for earthworm abundance [4]. Brassicaceae, such as mustard (*Sinapis alba* L.) or oilseed radish (*Raphanus sativus* subsp. *oleiferus* M.) have a low C:N ratio, as Fabaceae, but have been controversially discussed in terms of earthworm preferences and population size [3,4,25,26]. Similarly, controversial in the context of earthworms are Poaceae, e.g. oat (*Avena sativa* L.), which has a high C:N ratio, as this cereal has been reported as being preferred by earthworms in the field [4], but avoided under laboratory conditions [26]. It is not fully understood how C:N ratio and cover crops affect earthworm populations, however in general, cover crops and soil covered with vegetation appear to be more beneficial to earthworms than bare fallow [3,4,19].

The work described here set out to investigate relationships between cover crops and earthworms. Cover crop biomass production is a trade-off between food provision for earthworms, soil moisture and other not investigated ecosystem services like weed suppression and nitrogen leaching [27]. The field experiments were conducted in an area with a low annual precipitation (538 mm), therefore, an irrigation factor was included to secure plant development, as soil moisture is essential for biomass production and earthworm numbers. Due to findings of Euteneuer et al. [3], we hypothesised that radish, because of its low C:N ratio would support earthworm abundance especially under irrigation. Therefore, two seeding rates of radish were selected as this affects plant development [28], along with two Poaceae known for their different biomass gains [15,27]. Specific objectives were to: (i) assess the effects of selected cover crops on earthworm field populations under two irrigation regimes; and in the laboratory (ii) determine cover crop preferences of selected earthworms and (iii) measure growth rates and reproductive output of earthworms fed with selected cover crops.

## 2. Materials and methods

### 2.1. Field trials

#### 2.1.1. Experimental site

Field trials were conducted near the experimental farm of the University of Natural Resources and Life Science, Vienna in Gross-Enzersdorf (48°14′N 16°35′E; 156 m asl; Lower Austria, Austria) from July 2018 to April 2019. The experimental farm, located in the Pannonian Plain has an annual precipitation of 538 mm and an average temperature of 10.6 °C. The soil is a calcaric Chernozem [29] with $pH_{CaCl2}$ 7.6, and a soil depth of 60–90 cm with a field capacity of 0.32 $cm^3$ $cm^{-3}$ and a wilting point of 0.15 $cm^3$ $cm^{-3}$ [14].

#### 2.1.2. Experimental design and cover crops

Two randomised complete block design field trials were conducted, with plot size of 3 m × 10 m, to determine the effect of cover crops on earthworms. The two trials were identical, each with four replicates, adjacent in the field, but had different irrigation regimes. The first trial was rainfed and received in total 193.3 mm water from July to September 2018 and the second was additionally irrigated with 6 × 20 mm per week from August to the end of September (Table 1). Cover crops were sown in pure stands on July 18, 2018. Seed density of radish was chosen at high density (HD radish, *R. sativus* var. *longipinnatus* B. cv. 'Forza'; 250 seeds $m^{-2}$) due to a better covering of the soil surface and smaller tap roots, and low seed density (50 seeds $m^{-2}$; LD radish) to produce larger tap roots [28]. For both black oat (*A. strigosa* Schreb. cv 'Pratex'; 400 seeds $m^{-2}$) and Sudan grass (*Sorghum sudanese* M. cv. 'Piper'; 180 seeds $m^{-2}$) local standards were used and all cover crops were seeded in 12 cm row spacing with a drill seeder (Plot seeder S, Wintersteiger AG, Ried, Austria). Bare fallow was installed as a control and kept free from any plant biomass by hand-weeding. Experimental plots were installed when winter barley (*Hordeum vulgare* L.) residues were incorporated by a cultivator to a depth of 7 cm and after a second pass 10 days later.

**Table 1**
Continuously recorded air temperature (°C, monthly mean) and precipitation (mm, monthly cumulative) and additional irrigation (mm, monthly cumulative) for an irrigated field trial from June 2018 to April 2019. Irrigation was split into 20 mm $event^{-1}$ $week^{-1}$.

| Year | Month | Air temperature (°C) | Precipitation (+Irrigation) | |
|---|---|---|---|---|
| | | | Rainfed (mm) | Irrigated (mm) |
| 2018 | Jun | 21.0 | 93.5 | 93.5 |
| 2018 | Jul | 25.1 | 71.7 | 71.7 |
| 2018 | Aug | 26.4 | 22.8 (+20) | 22.8 (+80) |
| 2018 | Sep | 20.0 | 78.8 | 78.8 (+40) |
| 2018 | Oct | 15.0 | 0.4 | 0.4 |
| 2018 | Nov | 7.4 | 28.0 | 28.0 |
| 2018 | Dec | 3.5 | 70.0 | 70.0 |
| 2019 | Jan | 1.5 | 29.5 | 29.5 |
| 2019 | Feb | 6.1 | 8.9 | 8.9 |
| 2019 | Mar | 10.6 | 21.1 | 21.1 |
| 2019 | Apr | 13.3 | 0.0 | 0.0 |
| Total | | | 444.7 | 564.7 |

Cover crops remained in the field until April 17, 2019, when Sudan grass and black oat had been winter-killed by December 2018 and radishes by frost in January 2019. The aboveground cover crop production was measured in October 2018 by cutting 0.25 $m^2$ per plot at ground level with dry mass recorded after 24 h drying at 105 °C. Root biomasses for all treatments were taken with a sample auger (750 ml, depth 15 cm, $n = 1$) between the seeding rows and tap roots of radishes were excavated separately with 0.25 $m^2$ per plot, all roots were washed and processed similarly to aboveground biomass. C:N was determined by the Dumas combustion method (vario MACRO cube CNS; Elementar Analysesysteme GmbH, Germany) [30] after grinding and sieving (<1 mm). Chemical elements were analysed by inductively coupled plasma-optical emission spectrophotometry (iCap 7000 Series ICP-OES; Thermo Fisher Scientific, Waltham, USA) after nitric acid digestion of the material.

Soil moisture and soil temperature were measured on a weekly basis (0–7 cm depth; Delta-T Devices Ltd., Cambridge, UK; with calibration according to the manual) from August 2018 to the end of October 2018 and from the end of February 2019 to April 2019 (Fig. 1). Air temperature and precipitation was continuously measured for the whole trial period (Adcon A733, OTT Hydromet GmbH, Kempten, Germany; Table 1).

#### 2.1.3. Earthworms

Earthworm abundance was assessed by hand sorting of four soil monoliths (20 cm × 20 cm × 28 cm) per plot at October 16–23, 2018 and March 26 –April 1, 2019. Samples were packed in plastic bags for storage (4 °C) and searched for earthworms over the following four days. All earthworms were washed, classified to ecological group *sensu* Bouché [31] (viz.: epigeic, endogeic, anecic), counted and transferred to tissue paper to remove excess of water for mass recording. Adult earthworms were preserved in 70% ethanol and identified to species level according to Sherlock [32].

### 2.2. Laboratory experiments

#### 2.2.1. Experiment 1: food choice

To assess preference of cover crops by earthworms, two choice chamber experiments, with a set up similar to that of Rajapaksha et al. [23], were conducted with the plant material (partly winter-killed) collected from the Austrian field trials in December 2018, at the University of Central Lancashire (Preston, UK). In experiment 1a), aboveground plant biomass (shoots) and in 1b) belowground biomass (roots) were offered. From previous research [23], locally-collected birch leaves (*Betula pendula* R.) were used as a control in both experiments. From our field experimental findings of only endogeic earthworms and results of

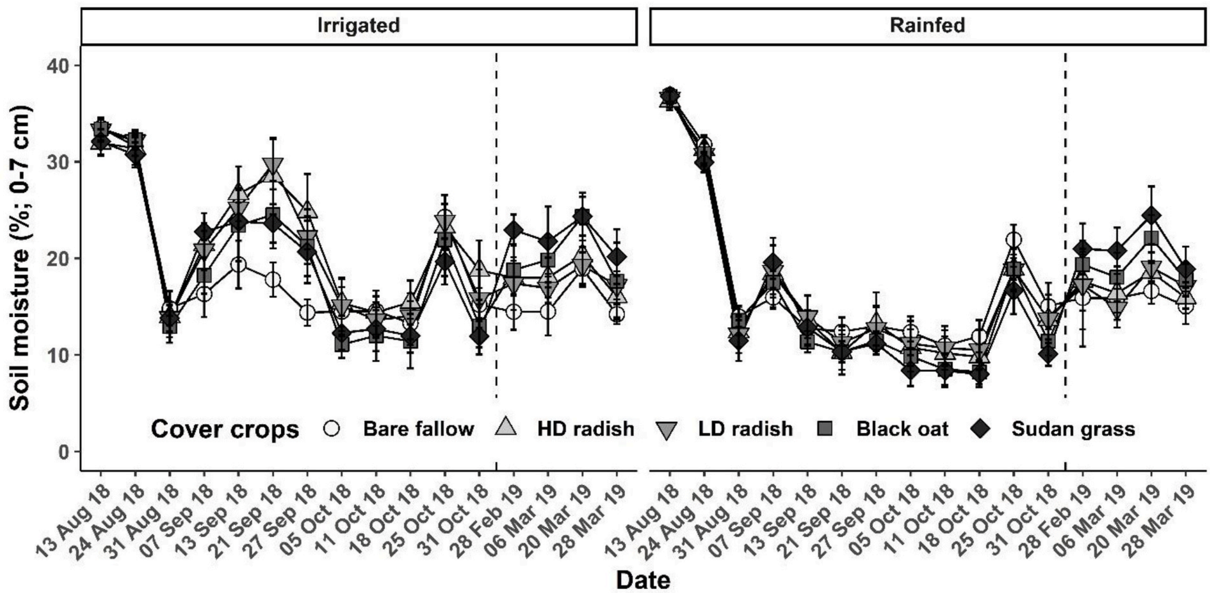


**Fig. 1.** Overview of soil moisture content (%) in the first 7 cm in two field trials (irrigated; rainfed) under four cover crop treatments (HD radish (high density), LD radish (low density), black oat, Sudan grass) and bare fallow in two growth periods, from August to October 2018 and from end of February to March 2019. Dotted lines indicates a sampling break between November 2018 and February 2019. Mean (±SD), $N = 4$.

Roarty et al. [4], we selected *Aporrectodea caliginosa* Sav. (endogeic). In addition, to broaden the species (ecotype) spectrum, we examined *Aporrectodea longa* Ude as a common (in the UK) anecic earthworm. All experiments used field-collected (53°42′N 2°40′W) adult earthworms that had been acclimated to laboratory conditions for three months prior to experimentation [33], when they were fed with a mixture of birch leaves and horse manure [22]. Initial individual mean masses were 0.55 ± 0.2 g for *A. caliginosa* and 2.55 ± 0.42 g for *A. longa*.

Each earthworm species was assessed separately, with either three *A. caliginosa* or two *A. longa* provided per choice chamber, which comprised of an aluminium foil tray (diameter 0.16 m and depth 0.03 m). Microcentrifuge tubes (diameter 0.01 m and depth 0.04 m) containing selected cover crop treatments were randomly arranged around each tray (total = 5; one per cover crop treatment plus control) with five replicate trays for each earthworm species. Cover crop preference was assessed by calculating mean loss of plant biomass in individual food tubes, measured every second day over 28 days. Trays were filled with Kettering loam with a moisture content of 25% [34] (Supplementary Fig. 1) and kept in darkness for 24 h at 15 °C in a temperature-controlled incubator. At the end of each experiment, the number of surviving earthworms and their masses were also recorded.

Cover crop biomass was dried at 105 °C for 24 h, ground separately (using knife mills, first GRINDOMIX GM 300 and subsequently GM 200, Retsch GmbH, Haan, Germany) and passed through a series of sieves (2.8, 2.0 and 1.0 mm). Cover crop particles (diameter 1–2 mm) were used to prevent undue influence of size on earthworm food selection. Before use, cover crop biomass was soaked with water for 2 h, and then tubes of known mass were filled to the same volume (0.63–1.82 g in each tube). Excess water was drained by inversion (30 min) on absorbent tissue paper and masses of wet food-filled tubes were recorded.

#### 2.2.2. Experiment 2: earthworm growth

Hatchlings of *A. caliginosa* or *A. longa* (mean individual masses of 22.4 ± 3.3 and 48.3 ± 5.7 mg, respectively) were obtained from laboratory-produced cocoons which had been incubated at 15 °C. Emerging hatchlings had been collected daily and kept in water at 5 °C until the required number was present. Plastic vessels of 0.4 L (depth 0.04 m) were filled with 150 g of moist soil (Kettering loam) and three hatchlings of either species were introduced into each vessel. The same processed cover crops (4 g) as used in experiment 1 were incorporated individually as food treatments with soil only as control. Experimental vessels were examined every two weeks and earthworm survival, mass and developmental stage were recorded before earthworms was returned to vessels. At four-week intervals, the substrate was replaced with fresh soil and feed. Four replicates per treatment were maintained and the experiment was terminated after 132 days.

#### 2.2.3. Experiment 3: earthworm reproduction

From the same field-collection, as described in section 2.2.1, three adult *A. caliginosa* ($n = 15$, mean individual mass 0.86 ± 0.16 g) were randomly assigned and kept in 0.75 L vessels (depth 0.1 m). Four cover crop treatments (HD radish, LD radish, black oat, Sudan grass) mixed into the soil, were used separately as feed treatments, with soil only (Kettering loam) as control. Experimental vessels were examined every 4 weeks. At sampling, earthworm survival, and mass change were recorded, before earthworms were re-provisioned with fresh soil and fed as before. Soil removed from vessels (on a 4-weekly basis) was wet-sieved through 2.0 and 1.0 mm meshes for collection of cocoons [34]. The experiment began in March 2019 and ended after 180 days.

### 2.3. Statistical analyses

Effect of cover crops over time, on earthworms and soil moisture on a field scale, were assessed using a three-way generalised linear mixed model (3-way GLMM), but only for pairwise comparison within the irrigation regimes (rainfed or irrigated) as these are considered as 2 separate trials. The fixed factors in this analysis were cover crop treatment (5 levels; control (Bare fallow), HD radish, LD radish, black oat, Sudan grass), irrigation (2 levels; irrigated vs rainfed) and time (2 levels each of: earthworms: October 2018 and March 2019; and soil moisture: summer to autumn and spring). GLMM were fitted with Quasipoisson distribution for earthworm counts (mean of the subsamples, $N = 4$), a Gamma distribution (link = inverse) for earthworm mass and for soil moisture (average per season; level 2; summer to autumn 2018 and spring 2019). The 'summer to autumn' season contains mean soil moisture % per cover crop treatment from August to October 2019 and the 'spring' season represents means from February and March 2019. The 3-way GLMM analyses were conducted with function 'glmmPQL' (package 'MASS') in R [35] using penalised quasi-likelihood (PQL). To model covariance among seasons, factors replicate, season and irrigation were set random with an autocorrelation structure of order 1 (function 'corAR1'; package 'nlme') for earthworms and a spatial correlation (corSpatial; package 'nlme') for soil moisture.

Cover crop plant biomass (shoots; roots), radish tap root biomass and plant C:N ratio (shoots; roots), were analysed by a linear model (2-way LM; function lm), with factors cover crop and irrigation and only for pairwise comparisons.

For food choice experiment 1, statistical analysis was performed by a three-way linear model (3-way LM) with fixed factors food (5 levels; control (Birch leaves), HD radish, LD radish, black oat, Sudan grass), plant part (2 levels; shoot vs root) and earthworm species (2 levels, *A. caliginosa* vs *A. longa*). To include the factor time (days), proportional differences between food mass loss per measurement (g $g^{-1}$; every second day) and initially provided food mass per treatment and replicate were summed (excluding day zero) and multiplied by two. A random effect was fitted per replicate, earthworm species and plant part to model covariance among different foods for the same earthworm species. Models were fitted by the function 'lmer' of the 'lme4' package with the residual maximum likelihood (REML) method. Furthermore, function 'Anova' was used to perform the analyses of variance (Wald-type *F*-tests using Satterthwaite's method for determining the denominator degrees of freedom and using type III hypotheses). Determination of changes in earthworm mass during the experiment was conducted with paired *t*-tests.

Earthworm mass gain in growth experiment 2 after 132 days was analysed in a two-way generalised linear model (2-way GLM) with factors food and earthworm species, with a Gamma distribution (link = inverse). Analysis was conducted by function 'glm' from the 'lme4' package and 'Anova' for analysis of deviance ($X^2$-value).

Nutrient content of cover crops was analysed similarly to the growth experiment in a 2-way GLM with factors food and nutrients, with Gamma distribution and pairwise comparison.

Cocoon counts and earthworm mass in experiment 3 were analysed with a generalised linear model (GLM and LM, respectively; *t*-value) with cumulative added cocoons over six months and final earthworm mass; both with factor food. For GLM, family = quasipoisson was used to determine the dispersion parameter (3.27).

All pairwise comparisons (Tukey test; $P < 0.05$) were computed by function 'emmeans' (package 'emmeans') with the relevant interactions and all significant pairwise comparisons described in the results section. Consolidated results are summarised with the lowest common *P*-value, individual results are given with the exact *P*-value. All data are mean values with related standard deviation (mean ± SD). Residual distribution and homogeneity of the variance were visually assessed by plotting frequency of residuals, box plots of residuals for each explaining variable and residuals vs fitted values per model, respectively. In GLMs overdispersion was checked by the deviance, divided by the residual degrees of freedom.

## 3. Results

### 3.1. Field trials

#### 3.1.1. Cover crops and soil moisture

Pairwise comparison of cover crop biomass in October 2018, for both trials, revealed no differences among HD, LD radish and black oat, only with Sudan grass (2-way LM; Tukey; $P < 0.05$; Table 2). Tap root biomasses of radishes were affected by seed density and tap roots of HD radish were smaller than LD radish (2-way LM; Tukey; $P < 0.001$; Table 2). Root biomass was not significantly different between the cover crops treatments (2-way LM; Tukey; $P > 0.05$; Table 2).

In the two seasons, soil moisture was affected by cover crops (Table 3) and pairwise comparison showed that black oat and Sudan grass under rainfed conditions had lower soil moisture from 'summer to autumn' than bare fallow (3-way GLMM; Tukey; $P < 0.05$). This was not seen under irrigation, when both HD and LD radish had the highest soil moisture (3-way GLMM; Tukey; $P < 0.05$). In spring, the former irrigation treatment had no influence on soil moisture, and below Sudan grass this was the highest recorded in both regimes (3-way GLMM; Tukey; $P < 0.05$).

**Table 2**

Results of statistical analyses (2-way LM; Tukey; $P < 0.05$) of plant biomass shoots (g $m^{-2}$), root biomass (g 750 $ml^{-1}$), radish tap roots (g $root^{-1}$) and plant C:N ratio (shoots; roots) in field trials with two irrigation regimes (rainfed; irrigated) and four cover crops (HD radish (high density), LD radish (low density), black oat, Sudan grass) in October 2018. Means having no letter in common (rainfed in lower case, irrigated in upper case) are significantly different by pairwise comparison (Tukey; $P < 0.05$). Mean (±SD), $N = 4$.

| Treatment | Plant properties | | | | | |
|---|---|---|---|---|---|---|
| | Rainfed | | – | Irrigated | | – |
| | Mean | SD | | Mean | SD | |
| | Plant biomass g $m^{-2}$ | | | | | |
| HD radish | 195 | 10.3 | b | 295 | 37.2 | B |
| LD radish | 174 | 86.3 | b | 295 | 118.8 | B |
| Black oat | 374 | 117 | b | 484 | 74.9 | B |
| Sudan grass | 912 | 76.8 | a | 799 | 315 | A |
| | Root biomass g 750 $ml^{-1}$ | | | | | |
| HD radish | 0.05 | 0.04 | a | 0.13 | 0.05 | A |
| LD radish | 0.10 | 0.14 | a | 0.19 | 0.03 | A |
| Black oat | 0.09 | 0.05 | a | 0.12 | 0.07 | A |
| Sudan grass | 0.12 | 0.10 | a | 0.19 | 0.08 | A |
| | Radish tap root (g $root^{-1}$) | | | | | |
| HD radish | 1.6 | 0.7 | b | 2.3 | 0.6 | B |
| LD radish | 6.6 | 1.7 | a | 8.4 | 0.3 | A |
| | C:N ratio plant biomass (shoots) | | | | | |
| HD radish | 14.4 | 2.31 | a | 17.1 | 5.22 | A |
| LD radish | 11.8 | 2.07 | a | 18.2 | 5.81 | A |
| Black oat | 45.8 | 12.03 | b | 35.6 | 3.54 | B |
| Sudan grass | 45.4 | 18.1 | b | 46.9 | 14.5 | B |
| | C:N ratio plant biomass (roots) | | | | | |
| HD radish | 11.4 | 3.1 | a | 16.5 | 4.11 | A |
| LD radish | 12.3 | 1.4 | a | 17.2 | 5.68 | A |
| Black oat | 44.7 | 10.6 | b | 39.01 | 8.84 | B |
| Sudan grass | 53.1 | 9.8 | b | 43.9 | 7.26 | B |

**Table 3**

Results of pairwise comparison (3-way GLMM; Tukey; $P < 0.05$) soil moisture (%) in field trials with two irrigation regimes (rainfed; irrigated) under four cover crop treatments (HD radish (high density), LD radish (low density), black oat, Sudan grass) and bare fallow in two seasons ('summer to autumn', spring). Means having no letter in common (rainfed in lower case; irrigated in upper case) are significantly different by pairwise comparison (Tukey; $P < 0.05$). Mean (±SD), $N = 4$.

| Treatment | Soil moisture % | | | | | |
|---|---|---|---|---|---|---|
| | Rainfed | | – | Irrigated | | – |
| | Mean | SD | | Mean | SD | |
| | 'Summer to autumn' | | | | | |
| Bare fallow | 17.4 | 8.3 | a | 19.1 | 6.9 | B |
| HD radish | 16.6 | 8.6 | ab | 22.2 | 6.8 | A |
| LD radish | 16.8 | 8.4 | ab | 21.6 | 7.4 | A |
| Black oat | 15.7 | 8.9 | b | 19.6 | 8.0 | B |
| Sudan grass | 15.3 | 9.0 | b | 19.7 | 7.2 | B |
| | 'Spring' | | | | | |
| Bare fallow | 15.9 | 2.8 | c | 15.5 | 2.7 | C |
| HD radish | 17.1 | 2.4 | bc | 18.1 | 2.3 | BC |
| LD radish | 17.1 | 3.1 | bc | 17.7 | 2.1 | BC |
| Black oat | 19.3 | 2.6 | ab | 20.2 | 3.7 | AB |
| Sudan grass | 21.3 | 3.2 | a | 22.3 | 3.0 | A |

#### 3.1.2. Earthworms

In October 2018 and March 2019, only endogeic earthworms were found, with adults in a proportion of 82% *A. caliginosa* to 18% *A. rosea* (March 2019). Overall mean of earthworm abundance in October 2018, for the trials, with and without irrigation, was 108.9 ± 30.6 and 81.0 ± 47.9 individuals $m^{-2}$, respectively (effect of irrigation between the field trials was not tested). In pairwise comparison under rainfed conditions, bare fallow had a higher earthworm abundance than black oat and Sudan grass (3-way GLMM: Tukey, $P < 0.01$; Fig. 2) and by March 2019, earthworm numbers had almost equalised between the irrigation regimes to 84.5 ± 31.5 and 78.3 ± 23.7 individuals $m^{-2}$ (irrigated and without irrigation, respectively; not tested).

No differences were detected in overall mean earthworm biomass in October 2018, neither under rainfed cover crops (bare fallow 35.8 ± 23.4 g $m^{-2}$, cover crops 12.6 ± 6.5 g $m^{-2}$) nor with irrigation (bare fallow 25.5 ± 9.1 g $m^{-2}$, cover crops 27.3 ± 12.9 g $m^{-2}$; Fig. 3). Earthworm biomass increased only for rainfed cover crops from October 2018 to March 2019, but not for bare fallow (2-way LM: Tukey; $P = 0.026$; overall means March 2019; rainfed: bare fallow 21.1 ± 17.4, cover crops 33.5 ± 17.2; irrigated: bare fallow 39.1 ± 13.0, cover crops 37.1 ± 19.1).

### 3.2. Laboratory experiments

#### 3.2.1. Experiment 1: food choice

Food choice was affected by cover crops, earthworm species and interactions of plant parts with earthworms (Table 4). *A. caliginosa* preferred roots of Poaceae and specifically black oat over the two radishes and control, whereas Sudan grass was selected before birch leaves and LD radish (3-way LM; Tukey; $P < 0.05$; Fig. 4). Feeding on shoots, *A. caliginosa* chose HD radish over birch and LD radish (3-way LM; Tukey; $P < 0.05$). However, *A. longa* fed more on cover crop roots than birch leaves and preferred LD radish rather than black oat roots (3-way LM; Tukey; $P < 0.05$). No preference for *A. longa* could be detected with shoots, but tubes of HD radish were emptied first, after 10 days, followed by LD radish at day 20. Over the course of the 28-day food choice experiments, most earthworms lost between 0.04 and 0.4 g in mass (paired *t*-test; $P < 0.05$), except *A. longa* fed on roots (paired *t*-test; $P = 0.2$). C:N ratio of plant biomass (shoots; roots) significantly differed between HD, LD radish (Brassicaceae) and black oat, Sudan grass (Poaceae; LM; Tukey; $P < 0.05$; Table 2).

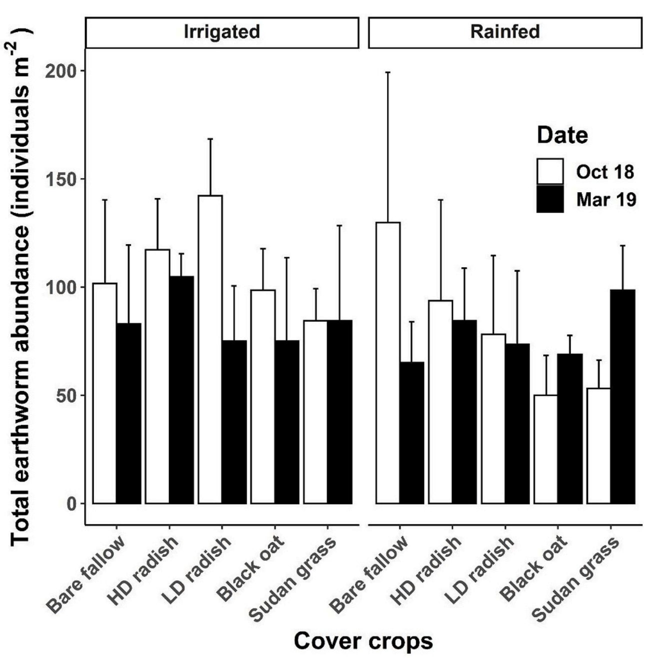


**Fig. 2.** Earthworm abundance (individuals $m^{-2}$) in two field trials with different irrigation regimes (irrigated; rainfed) under four cover crop treatments (HD radish (high density), LD radish (low density), black oat, Sudan grass) and bare fallow in October 2018 and March 2019. Adult earthworm composition is 82% *Aporrectodea caliginosa* and 18% *Aporrectodea rosea.* Mean (±SD), $N = 4$.

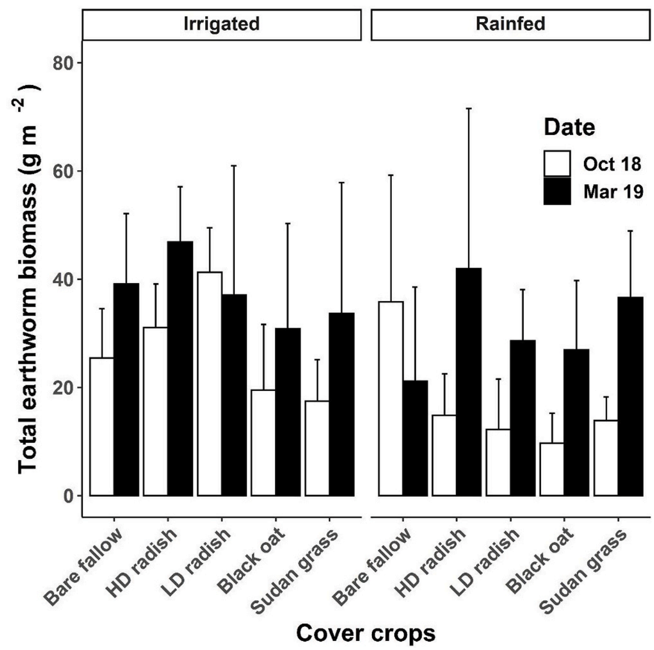


**Fig. 3.** Earthworm biomass (g $m^{-2}$) in two field trials with different irrigation regimes (irrigated; rainfed) under four cover crop treatments (HD radish (high density), LD radish (low density), black oat, Sudan grass) and bare fallow in October 2018 and March 2019. Mean (±SD), $N = 4$.

**Table 4**
Results of statistical analyses (3-way LM) of food preferences for two earthworm species (*Aporrectodea caliginosa* and *Aporrectodea longa*) in laboratory experiments with five food types (Birch leaves (control), HD radish (high density), LD radish (low density), black oat, Sudan grass) and two plant parts (shoots vs roots). Degrees of freedom (Df), *F*-value, $N = 5$.

| Treatment | Df | *F* value | |
|---|---|---|---|
| Food (F) | 4 | 493.3 | *** |
| Earthworm species (E) | 1 | 83.0 | * |
| Plant part (P) | 1 | 0.0 | |
| F × E | 4 | 124.3 | *** |
| F × P | 4 | 135.7 | *** |
| E × P | 1 | 25.3 | |
| E × F × P | 4 | 0.9 | |

* Significance level:<0.05.
*** Significance level:<0.001.

#### 3.2.2. Experiment 2: earthworm growth

With all cover crops, *A. caliginosa* gained equally in mass, but remained at an initially low level for the soil only (control) treatment (2-way GLM; Tukey; $P < 0.001$; Fig. 5). The same appeared for control and cover crops with *A. longa*, but this species reached higher masses with LD radish compared to black oat (2-way GLM; Tukey; $P < 0.01$). Therefore, earthworm species are differently affected by provided food (ANOVA; $X^2 = 15.9$, $P = 0.011$). The first tubercula pubertatis were recorded for *A. caliginosa* at day 119 in LD ($n = 2$) and HD radish ($n = 1$). At the end of the experiment, after 132 days, one *A. caliginosa* in each of LD, HD radish and black oat was clitellate and *A. longa* developed tubercula pubertatis in LD ($n = 3$) and HD radish ($n = 3$).

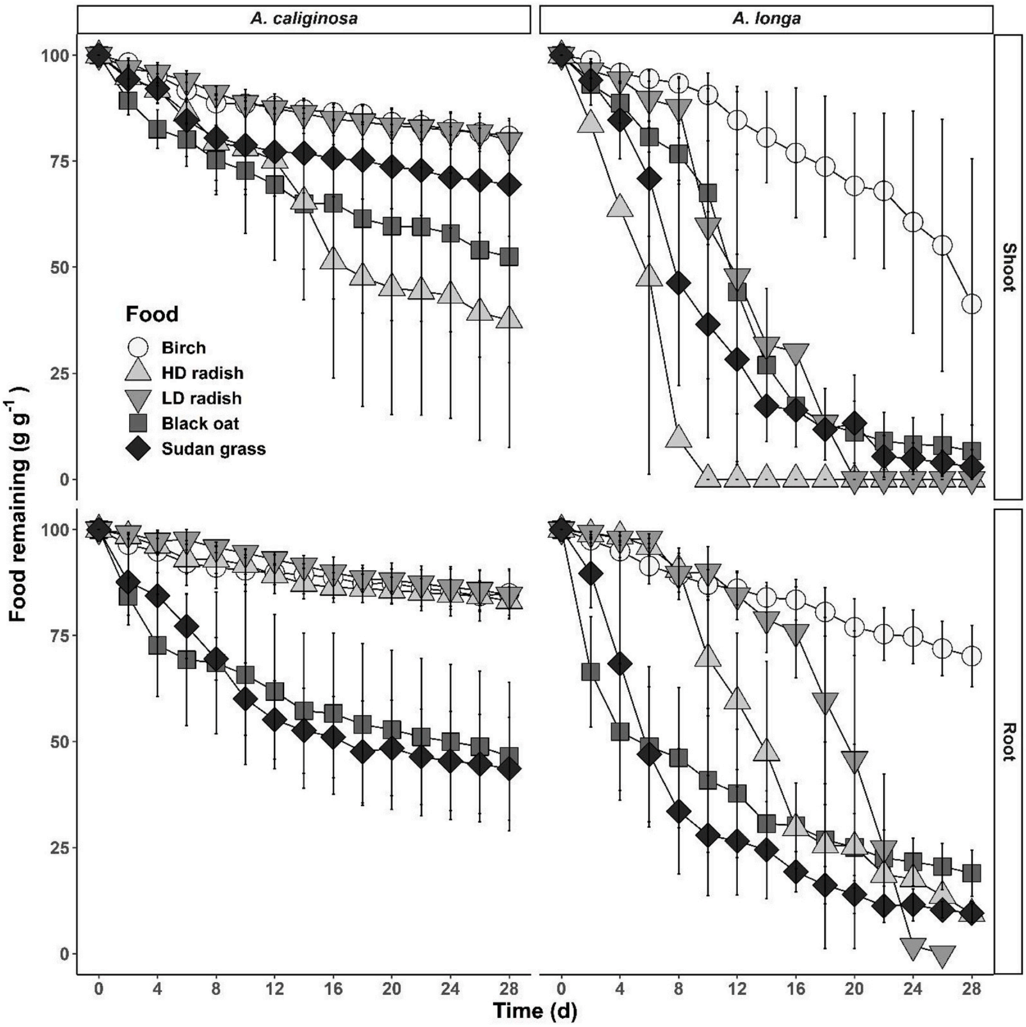


**Fig. 4.** Proportion of food remaining (g $g^{-1}$) for two earthworm species (*Aporrectodea caliginosa* and *Aporrectodea longa*) in laboratory experiment 1 with five food sources (Birch leaves (control), HD radish (high density), LD radish (low density), black oat, Sudan grass) and two plant parts (shoots, roots) over 28 days. Mean (±SD), $N = 5$.

#### 3.2.3. *Experiment 3: earthworm reproduction*

Cocoon production of *A. caliginosa* differed significantly between the control and the two brassica crops and the grass crops (GLM; Tukey, $P < 0.001$; Fig. 6). More cocoons were produced when fed with HD radish compared with black oat (GLM: Tukey; $P = 0.028$), and overall highest number of cocoons was recorded in vessels with LD radish compared to Sudan grass and black oat (GLM: Tukey; $P < 0.05$). No differences were recorded in earthworm masses gained between cover crops themselves, only between cover crops and control (LM: Tukey, $P < 0.001$).

Nutrient analyses of cover crops revealed higher Ca (calcium), Fe (Iron) and Mg (magnesium) contents for both radishes compared with grass crops (2-way GLM; Tukey, $P < 0.05$; Supplementary Table 1).

## 4. Discussion

### 4.1. *Field trials*

#### 4.1.1. *Cover crops, soil moisture and earthworms*

In field trials, we aimed to elucidate the effect of cover crops as biostimulators on the abundance of earthworms. The different cover crops provided food, coverage of the soil surface and influenced soil moisture [4,13,19,24,36,37]. Radish tap roots differed in size, but similarity in shoot biomass of LD and HD radish in both irrigation systems illustrated plant plasticity under low and high seed densities [28]. Sudan grass, as a $C_4$ plant, had a higher water efficiency and highest biomass of the cover crops used [38]. Due to an extended root system, Sudan grass extracts water more effectively [39,40]. Therefore, Sudan grass is more suitable for cover cropping under dry conditions but could increase water stress for earthworms during summer and autumn [18, 38].

Soil moisture was affected by cover crops in terms of plant biomass according to water usage for plant growth, shading of the soil surface and dew formation [13,41], but not plant density. Transpiration of cover crops decreased soil moisture until October 2018 and especially with Sudan grass and black oat [13,38]. These species had higher C:N ratios than radishes and only slowly decomposed over winter. Hence, the Poaceae covered the soil against evaporation more effectively than radishes until spring [13]. Furthermore, plant height can increase precipitation due to dew formation [41] and might have induced higher soil moisture in spring with Sudan grass and black oat and thereby almost equalised the earthworm populations between the treatments [17,18]. This is in line with findings of Abail and Whalen [42], where high vs low plant residues of maize (stems, tassels, cobs, roots) supported the mainly endogeic earthworm population, compared with soybean residues with a lower lignin and C content. Abail and Whalen [42] concluded, that earthworm abundance was not affected by lignin, but by the amount of residues, which might have also affected soil moisture, temperature and other physical and biotical attributes. In addition, Abail and Whalen

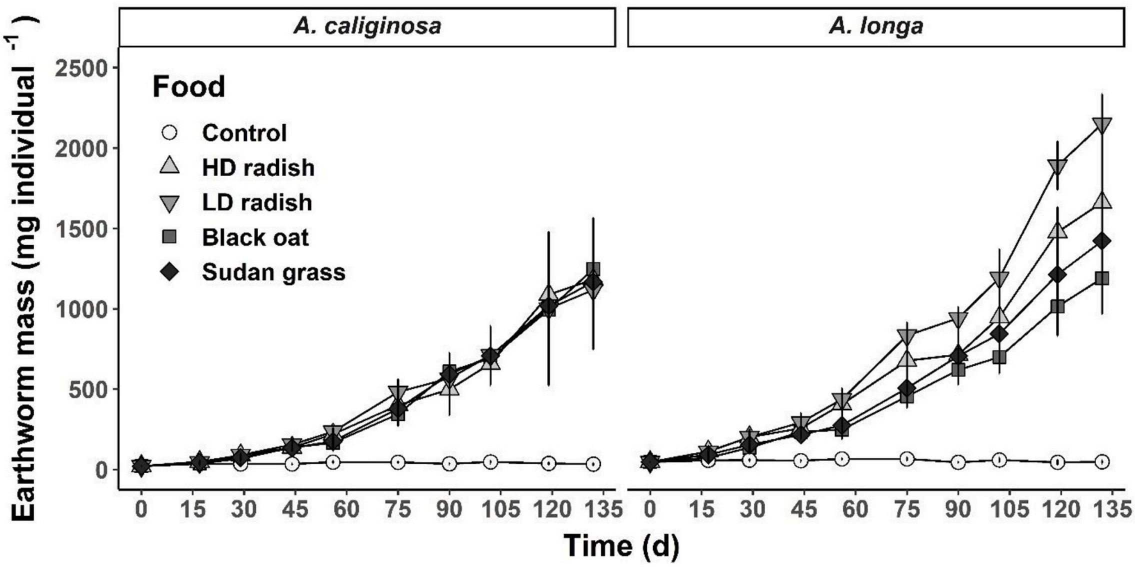


**Fig. 5.** Earthworm mass gain (mg individual$^{-1}$) for two species (*Aporrectodea caliginosa* and *Aporrectodea longa*) in laboratory experiment 2 with cover crop treatments and a control (soil only (control), HD radish (high density), LD radish (low density), black oat, Sudan grass) over 132 days. Only aboveground plant biomass shoots were used. Mean (±SD), $N = 5$.

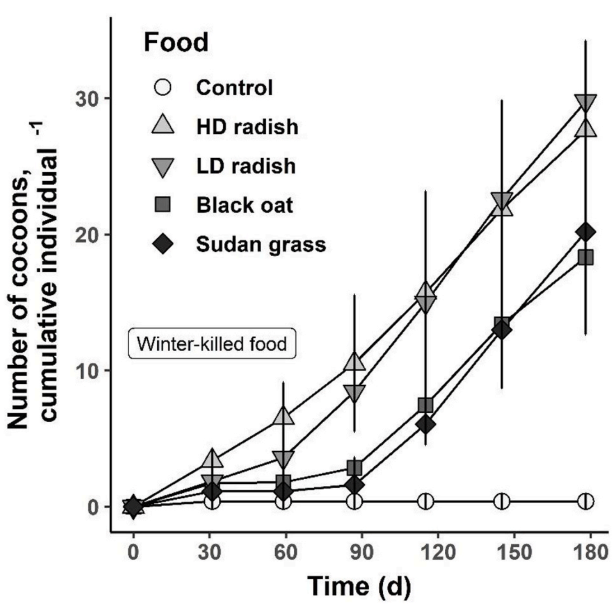


**Fig. 6.** Cumulative number of earthworm cocoons produced per individual of *Aporrectodea caliginosa* over 178 days in laboratory experiment 3 with five food sources (soil only (control), HD radish (high density), LD radish (low density), black oat, Sudan grass). A change of food occurred after day 31, when autumn samples of aboveground plant biomass was replaced by spring sampled (winter-killed) plant biomass. Mean (±SD), $N = 5$.

[42] stated that after 11 months decomposition in the field, maize residues became a palatable and sustainable food resource for a growing earthworm population.

In our field trial, only earthworm biomass (rainfed cover crops) increased from autumn to spring, but not necessarily earthworm numbers. Our findings are incongruent with previous works, where earthworm populations and biomass increased over winter and particularly under oilseed radish and pea [3,4]. This discrepancy may be explained by the fact that, in October 2018, soil moisture was lower than 20% and many juvenile earthworms were found in aestivation and may have died before adequate rainfall for activity in December 2018. In rainfed conditions, bare fallow had highest earthworm numbers and soil moisture in October 2018. Hence, in the short-term, earthworm abundance was directly affected by soil moisture, more than by cover crops, but over winter earthworm numbers equalised and biomass was increased [4,17–19,42]. Our hypothesis that radish increased earthworm abundance could not be verified, but it was seen that over winter, cover crops supported earthworm biomass under required soil moisture conditions.

### 4.2. Laboratory experiments

#### 4.2.1. Food choice

Earthworm species showed differing food preferences for plant parts in the food choice test, but generally no clear pattern could be found for selected C:N ratios as in previous studies [3,4,19,22,25,43,44]. Enough N content of food is necessary for earthworm growth and cocoon production [22], but for choice, other food properties, such as secondary plant products and food palatability may be more important [42–46]. It is therefore thought that *A. caliginosa* fed on lignocellulosic black oat and Sudan grass roots with a higher C:N ratio rather than radishes, as similar was seen for *Lumbricus terrestris* L., fed with Italian ryegrass (*Lolium multiflorum* Lam.) and mustard by Valckx et al. [26]. Furthermore, *A. caliginosa* and *A. longa* were reported [43,44] to be among the less selective of earthworms, but we have seen an interaction of cover crop and plant parts for *A. longa* and clear food preferences of *A. caliginosa*. Both radish shoots were the first to be removed and LD radish root was significantly favoured by *A. longa,* in addition to HD radish shoot by *A. caliginosa,* which still emphasises the importance of nitrogen content in food [20,23,43]. Nevertheless, in the food choice test we see our hypotheses confirmed, in that earthworms preferred radishes over Poaceae as the former were removed first in three of the four food choice experiments.

Laboratory-based results from provision of plant material to geophagous species such as *A. caliginosa* may be questionable, due to their known feeding behaviour in the field [42,47]. Nevertheless, these species were free to select in food choice tests, which in combination with growth and reproduction experiments, provide insights into short-term responses of earthworms to specific cover crop residues. Ongoing field experiments consider investigation of long-term effects of cover crops on earthworm populations after plant residues have been incorporated and decomposed, as *A. caliginosa* have been found to feed on material associated with soil and old carbon pools [47,48].

*4.2.2. Earthworm growth and reproduction*

*A. longa* hatchlings fed with radishes were positively affected by higher N content in respect of development stage and mass gain, as previously seen with *L. terrestris* [22]. In contrast to Boström [49], mass gain of *A. caliginosa* was unaffected by C:N ratio, however, development stage and cocoon production were impacted by radishes in accordance with Boström [49]. The cocoon production of *A. caliginosa,* with respect to offered cover crops, varied between 2.1 and 3.5 cocoons individual$^{-1}$ week$^{-1}$ and was slightly higher than number of cocoons observed by Bart et al. [50,51] and Boström [49] at 1.0, 2.4 and 1.3 cocoons individual$^{-1}$ week$^{-1}$, respectively. According to Bart et al. [51] these different findings may be related to the amount of food provided per individual. Radishes are higher in Ca and Mg (and Fe) concentration, which increase earthworm growth [19,51–53] as was shown for *A. longa*, but not for *A. caliginosa*.

### 4.3. Future work

To meet the requirements of sustainable agriculture in relatively dry areas, further research is necessary to elucidate divergent demands of soil moisture by plants and earthworms. A trade-off is needed to satisfy their different ecosystem functions and services, such as a high plant biomass and weed suppression or high earthworm numbers under low precipitation. Therefore, investigation of mixed cover crops may be warranted, to determine the most beneficial composition for enhanced earthworm communities. Such investigations could usefully employ variation in cover crop combination, plant density and levels of irrigation in addition to their legacy effect in following cash crops. Laboratory work could consider comparative trials, feeding earthworms with roots, shoots, with and without mycorrhiza colonisation and include secondary plant products such as soluble sugars. These might also be offered in excess to avoided potential resource depletion.

## 5. Conclusion

The feeding preference of both earthworm species (*A. caliginosa* and *A. longa*) clearly favoured radish over Sudan grass and black oat and therefore confirmed the importance of a lower C:N ratio for earthworm growth and reproduction. Cover crops in adequate soil moisture conditions, favoured earthworm abundance in the field trial. In addition, cover crops of high C:N ratio with a slow decomposition rate increased soil moisture and earthworm abundance over winter. A mixture of plant species with different C:N ratio is therefore preferable, than sole crops as both structure and food is needed to enhance earthworm abundance. In the long-term, cover crops supported earthworm populations compared to bare fallow which emphasises the significance of biostimulation of earthworms via agricultural managing practices.

## Author contributions

Conceptualisation: Euteneuer, P., Butt, K.R., Wagentristl, H.; Methodology: Euteneuer, P., Butt, K.R., Zaller, J.G., Piepho, H.-P.; Formal analysis and investigation: Euteneuer, P., Butt, K.R., Fuchs, M.; Writing - original draft preparation: Euteneuer, P.; Writing - review and editing: Euteneuer, P.; Funding acquisition: Euteneuer, P.; Resources: Wagentristl, H. Supervision: Steinkellner, S., Butt, K.R.

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## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The research was partly supported by SAATBAU LINZ eGen (Leonding, Austria).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2020.103225.

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