# Materials and Methods

## Field management

This study was conducted within a larger long-term crop rotation and tillage experiment which was established in 2002 on a sandy loam at Flakkebjerg Research Centre, Denmark (55.317, 11.400) and is still running. Averaged across the trial site, soil texture is 14.7% clay (<2 mm), 13.7% silt (2-20 mm), 42.6% fine sand (20-200 mm), and 27% coarse sand (200-2000 mm), with 1.2% organic carbon content (0-25 cm). The overall experimental design is a split-split plot with four replications. The main plot factor is cropping system (four levels), the sub-plot is primary tillage system (four levels) and the sub-sub-plot (six levels) was established to accommodate various sub-treatments within rotation and tillage combinations (see for example Melander et al. (2008)).

For the purpose of the present study, two cropping systems were selected that had the same sequence of crops but with different straw management: straw removed or retained. The cash crop sequence during the present study was spring barley (*Hordeum vulgare* L.) sown 19 April 2018, spring oat (*Avena sativa* L.) sown 4 April 2019 and faba beans (*Vicia faba* L.) sown 15 April 2020. Therefore, in the present study, straw management composed the main-plot treatment. Tillage sub-plots were 5 meters wide and 40 m long. Each tillage sub-plot was divided into two columns with three sub-subplots arranged within each column for a total of six sub-sub-plots that were 2.5 m wide and 12.5 m long (see supplementary material for a visual aid). One of the six sub-subplots was reserved for other sampling efforts, and cover crop system (five levels) was applied to the remaining five. In summary, the experiment included all combinations of two straw-managements, three primary tillage systems, and five cover crop systems for a total of 30 treatments.

The same straw managements and categorical tillage system (no tillage, non-inversion, inversion) have been in the same sub-plots since 2002. In the straw-removal treatments, following harvest of a small grain the residue (e.g., straw) was removed using a MACHINE, resulting in removal of approximately X% of the biomass (do we have any pictures of the straw retained and straw removed treatments, so we can see if the ground was still covered by little bits of straw after straw removal?) before any tillage was performed. In the straw-retained treatments, harvest residue was allowed to remain in the field. The exact machinery and timing of operations for each categorical tillage treatment have varied since 2002 (Scherner et al., 2016; Hansen et al., 2010). For the timeframe of the present experiment (2018-2020) a Horsch Terrano 3 FX stubble tine cultivator was used for the non-inversion tillage system,applying one pass just after cash crop harvest to 3-4 cm soil depth and then again just before cash crop sowing to 8-10 cm soil depth . For the inversion tillage system, before autumn-sown crops the soil was moldboard ploughed to 20 cm soil depth, followed by seedbed harrowing to 3-4 cm soil depth. In the case of spring-sown crops the moldbord plowing occurred in the fall, but seedbed harrowing was done in the spring before cash crop planting. Glyphosate, (Roundup Bio, 360 g a.i. l-1, Monsanto) at a dose of 540 g a.i. ha-1 was used in the no tillage and non-inversion tillage treatments to kill weeds and volunteer crop plants prior to drilling of cash crop. In the no tillage and non-inversion tillage systems, crops were sown with a chisel coulter (Horsch Airseeder CO 3) with row spacings of 17.5 cm for spring oats, spring barley, and faba beans. In the inversion tillage system, crops were sown with a traditional seed drill (Nordsten Lift-o-matic CLH300) after seedbed harrowing with row spacings of 12.5 cm. Why are the row spacings different in the moldboard plowing system?

Starting in 2018, five cover crop systems were randomly applied to the sub-sub-plots (**Table 1).** The same sub-sub-plot treatments were maintained for 2018 and 2019. The sampling area for all measurements was located in the inner 1.5 m x 10 m area of the sub-sub-plots.

Table 1. Summary of the five cover crop systems

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| Cover crop system name | Cover crop | Seeding rate | Seeding method and timing |
| Mix-early | Perennial ryegrass *(grass; Lolium perenne)* and red clover *(clover; Trifolium pratense)* | 3 kg ha-1 grass + 8 kg clover ha-1 | Sown 1 cm deep in 12.5 cm rows between cash crop rows shortly after cash crop planting |
| Mix-mid | Broadcast into standing crop approx. 14 days before expected cash crop harvest |
| Radish-mid | Fodder radish *(Raphanus sativus)* | 14 kg ha-1 | Broadcast into standing crop approx. 14 days before expected crop harvest |
| Radish-late | Broadcast into the crop stubble post crop harvest |
| No CC | - | - | - |

The plots planned for spring barley were fertilised 17 April 2018 with a mineral fertiliser using 126 kg N ha-1, 24 kg P ha-1 and 60 kg K ha-1. Plots planned for spring oat received 80 kg N ha-1, 15 kg P ha-1 and 38 kg K ha-1 on 3rd April 2019 using a mineral fertiliser. Finally, faba beans was fertilised with a mineral fertiliser on 15 April 2020 (same day as sowing) using the rates 32 kg P ha-1 and 80 kg K ha-1.

In 2018 (spring barley), plots except the Mix-early plots were sprayed for weeds on 16 May 2018 [0.25 l ha-1 Starane 333 HL (83 g a.i. Fluroxypyr) plus 0.03 I ha-1 Hussar OD (3 g a.i. Iodosulfuron) plus 0.5 Renol l ha-1]. To accommodate the presence of the grass and clover in the Mix-early plots, those plots were sprayed on 16 May 2018 with a different herbicide package that does not affect clover or grasses [12 g Harmony SX (6 g a.i. Thifensulfuron-methyl) + 0.15 l agropol ha-1]. On 29 May 2018 all plots except the Mix-early plots were sprayed with herbicide [1 l ha-1 MCPA Metaxon (750 g a.i. MCPA)] to control to control Canada thistle (*Cirsium arvense*).

In 2019 (spring oat), on 14 May all plots except the Mix-early plots were sprayed for weeds [0.5 l ha-1 Starane XL (90 g a.i. Fluroxypyr) plus 10 g ha-1 Trimmer SG (5 g a.i. Tribenuron-methyl) plus 0.15 l Agropol ha-1]. The Mix-early plots were sprayed with an herbicide package that does not impact clover or grasses [12 g Harmony SX (6 g a.i. Thifensulfuron-methyl) + 0.15 l Agropol ha-1].

In 2020, all faba bean plots were sprayed on 6 May 2020 and again on 20 May 2020 with herbicides [0.5 l ha-1 Stomp CS (228 g a.i. Pendimethalin) plus 0.4 l ha-1 Fighter 480 (192 g a.i. Bentazon)]. On 2 June 2020, wild oat (*Avena fatua*) was controlled with 0.93 l ha-1Agil 100 EC (93 g a.i. Propaquizafop).

All plots were managed identically for diseases and insect pests according to Danish standard recommendations and policies.

The long-term experiment is rainfed, but in 2018 an exception was made due to an exceptional hot and dry growing season. In order to maintain the long-term viability of the experiment, all plots were irrigated with 25 mm in early June to ensure the early establishment all treatments. Irrigation was done with sprinklers mounted on a boom that was dragged through the experiment.

## Measurements

Timing of measurements relative to other field activities is presented visually in supplemental material.

### Weather

### Weather data for the present study was obtained from the Danish Meteorological Institute's (DMI) Open Data API for the Flakkebjerg station (55.322, 11.388). The 30-year (1990-2020) mean annual temperature and precipitation for the site are 8.9 degrees Celsius and 589 mm, respectively. Crop yields

Each plot (net size 10 m x 1.5 m) was harvested for grain yield with a plot combine (8 August 2018 barley, 15 August 2019 oat, 24 August 2020 faba bean). Dry matter content was determined by a near-infrared spectroscopy analyzer (Infratec™ 1241 Grain Analyzer, Foss A/S; [Buchmann et al., 2001](https://www-sciencedirect-com.ez.statsbiblioteket.dk/science/article/pii/S0167198710000541" \l "bib4)). Grain yields are reported on a dry matter basis.

### Fall ground cover composition

Ground cover composition was estimated from digital images taken in the fall (9 November 2018 and 1 November 2019) as done in Melander et al. (2013). A 0.5 m2 quadrat was placed in the plot, and an image was taken from a height of 1 m above the center of the quadrat. Three images were taken in each plot. Each image was subsequently overlaid with a grid consisting of 17 vertical and 17 horizontal lines, resulting in 289 intersections per image. Each intersection was classified as a soil or plant. Plant intersections were identified to the species level (Table 1). Percent coverage of each species in the quadrat was then calculated by dividing the number of touched intersections in that category by 289 intersections. For categorical analyses, species were subsequently classified as ‘cover crop’ or ‘other’, depending on the plot treatment. For example, a *Lolium perenne* intersection was classified as ‘cover crop’ in the Mix-early and Mix-mid plots, but as ‘other’ in all other plots.

### Fall biomass

The amount of aboveground vegetative biomass in each treatment was measured following image collection for fall ground cover measurements (15 November 2018 and 13 November 2019, respectively). Two randomly placed 0.5 m2 quadrats were randomly placed in each plot, and all aboveground biomass was cut at ground level and removed. The biomass samples were separated into three fractions: cover crop, weeds and volunteers in 2018. In 2019, only the fractions cover crop and weeds (weeds plus volunteers) were obtained. The fractions were dried in the oven at 80oC for 24 hours and weighed. Dry biomass for each category was converted to grams per m2.

### Spring weed counts

The weed flora emerging in spring in the experimental plots was assessed on 22 May 2019 and 27 May 2020 by counting four weed categories in three randomly placed quadrats (0.25 m2) per plot. The categories were dicots, monocots, Canada thistle shoots and shoots from horsetail (Equisetum arvense). The spring counts were made to record whether there were any traceable effects from previous year’s cover crop treatments. (The weed counts in spring were of course affected by the earlier herbicide spring applications. The perennials were not affected, and dicots and monocots were not completely killed by the time of weed counting when a sulfonylurea product had been used as in 2019. In Faba beans, however, more dicots had been affected by the time of weed counting but not the monocots and shoots from perennials. I will postulate that strong cover crop effects from previous year would have been traceable on the following weed flora in spring despite the blurring/masking effect of chemical weed control).

Table X. Summary of vegetation measurements

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| **Measurement** | **Units of identification** |
| Fall ground cover (%) | Soil  Species (see supplementary material for complete list) |
| Fall biomass (g m-2) | Cover crop  Volunteer (according to previous crop)  Other (all other biomass) |
| Spring weed counts (number m-2) Bo, making sure the data you gave me is on a per m2 basis | Cirsium arvense  Equisetum arvense  Dicot  Monocot |

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