# Materials and Methods

## Field management

This study was conducted within a larger 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 site, soil texture was 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).

Weather data 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.

The overall experimental design was a split-split plot with four replications. The main plot factor is crop rotation (four levels) and the sub-plot is primary tillage (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 this study, two crop rotations were selected that had the same sequence of crops but with different straw management: straw removed or retained . The cash crop sequence during this 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. So in this study, straw management composed the main-plot and the sub-plots consisted of three randomly assigned primary tillage treatments within each main plot. Subplots 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. One sub-subplot was reserved, resulting in two straw treatments, three tillage treatments, and five cover crop treatments for a total of 30 treatments. A visual aid representing the experiment is available in supplemental material.

The same straw managements and categorical tillage treatments (no tillage (D), non-inversion tine tillage (H8-10), and inversion tillage (P)) have been in the same sub-plots since 2002, but the exact machinery used to achieve each tillage treatment has varied over the years (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 tillage treatment H8-10 applying one pass just after harvest to 3-4 cm soil depth and then again just before cash crop sowing to 8-10 cm soil depth . Moldboard ploughing to 20 cm soil depth followed by seedbed harrowing to 3-4 cm soil depth was used for treatment P and took place just before the autumn-sown crops were sown, and in the case of the spring-sown ones in late autumn with seedbed harrowing in spring. Glyphosate, (Roundup Bio, 360 g a.i. l-1, Monsanto) at a dose of 540 g a.i. ha-1 was used in all the non-inverted tillage treatments to kill weeds and volunteer crop plants prior to drilling.

The crops were sown with a chisel coulter (Horsch Airseeder CO 3) in D and H8-10 and with a traditional seed drill (Nordsten Lift-o-matic CLH300) in P after seedbed harrowing. Tillage treatments D and H8-10 were sown at the same row distance of 17.5 cm while in P crops were established at 12.5 cm inter-row spacing.

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

Table X. Summary of the five cover crop treatments applied in this experiment

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

The plots planned for spring barley was 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.

Spring barley was sprayed against weeds with 12 g Harmony SX (6 g a.i. Thifensulfuron-methyl) + 0.15 l agropol ha-1 in the plots on the 16th may where grass + clover was established right after sowing in early spring (the herbicide does not affect clover and grasses). All other plots were sprayed with 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 on 16th May. Later all plots, except for the plots with early establishment of grass+clover, was sprayed with 1 l ha-1 MCPA Metaxon (750 g a.i. MCPA) on 29 May, mainly to control Canada thistle.

Spring oat was sprayed against weeds with 12 g Harmony SX (6 g a.i. Thifensulfuron-methyl) + 0.15 l Agropol ha-1 in the plots on the 14th May 2019 where grass + clover was established right after sowing in early spring (the herbicide does not affect clover and grasses). All other plots were sprayed with 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 on 14th May 2019.

Faba beans was sprayed against weeds with 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 6 May 2020 and again on 20 May using the same dosages at both dates. Later, on the 2nd June, wild oat was controlled with 0.93 l ha-1Agil 100 EC (93 g a.i. Propaquizafop).

Diseases and insect-pests were controlled with pesticides according to Danish standard recommendations.

Crops in the long-termed CENTS experiment are rainfed but an exception was made in 2018 due to an exceptional hot and dry growing season. All plots were irrigated with 25 mm in early June to ensure the early establishment of grass + clover (as said all other plots were irrigated as well to avoid a crop growth bias caused by differences in water supply). Irrigation was done with sprinklers mounted on a boom that was dragged through the experiment.,.

## Measurements

### Crop yields

Each plot (net size 10 m x 1.5 m) was harvested for grain yield with a plot combiner (harvest dates: barley 8 August 2018, oat 15 August 2019 and beans 24 August 2020). 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)) on which also protein content in the cereals was determined. Grain yields are adjusted and presented with 15 % moisture content.

### Vegetation measurements

Three vegetation measurements were associated with each cover cropping phase (**Table X**). Following the cash crop harvest fall ground cover, fall biomass, and spring weed counts were measured and associated with the 2018 and 2019 cover cropping seasons, respectively.

Table X. Summary of vegetation measurements

|  |  |
| --- | --- |
| **Measurement** | **Units of identification** |
| Fall ground cover (%) | Soil  Cover crop/species (according to treatment)  Other/species or genus (see supplementary material) why are these to Cirsium species, and spring weed counts are only Cirsium arvense? All thistle counts relate to Cirsium arvense. |
| Fall biomass (g m-2) | Cover crop (according to treatment)  Volunteer (according to previous crop)  Other (all other biomass) |
| Spring weed counts (number m-2) | Cirsium arvense  Equisetum arvense  Dicot  Monocot |

Timing of measurements relative to other field activities is presented in **Figure X1**.

A screenshot of a computer

Description automatically generated

Figure X1. Timeline of field activities and sampling events. Non-inversion tillage consisted of…(I’m not sure) early spring harrowing and chisel plowing, inversion tillage of mold-board plow

#### 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 100 cm 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 genus or species level (Table X), and classified as ‘cover crop’ or ‘other’, depending on the plot treatment. For example, a *Lolium perenne* intersection was classified as ‘cover crop’ in plots with an *L. perenne*-*Trifolium repens* cover crop mixture, but as ‘other’ in all other plots. Percent coverage of each category in the quadrat was then calculated by dividing the number of touched intersections in that category by 289 intersections.

#### Fall biomass

The amount of biomass produced by the cover crops was assessed by the end of the growing season by cutting all aboveground biomass at ground level in two 0.5 m2 randomly placed quadrats per plot on 15 November 2018 and 13 November 2019. The 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 to obtain dry matter content.

#### 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).

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