LegacyNet Protocol
(Version 14, date published 02/11/2023)

This document provides an overview of measurement variables, and step by step instructions for the planning and establishment of the LegacyNet experiment at individual sites.

The LegacyNet website is: https://legacynet.scss.tcd.ie/. Contact details for the LegacyNet Directors are available at: https://legacynet.scss.tcd.ie/leaders-members.php

Contents

Summary of the changes from earlier versions of the protocol.

Version	Section	Notes
Version 1 to 2	Section 2.3	Some of the column labels in Table 1 have been amended to match the new data recording template.
	Section 2.5	The guidelines for the application of fertiliser have been clarified.
	Section 2.5.1	This section has been added to clarify the visual assessments of species proportions prior to each harvest. Subsequent subsections 2.5.2 and 2.5.3 are thus renumbered.
	Section 2.5.2	Some clarifications have been added to the text here, and to Figure 2.
	Section 2.6	An error in the first sentence has been corrected; previously it stated that follow-on crop should be sown "at least 18 months after the first harvest", which was incorrect, it should have been "at least 18 months after establishment".
	Section 3	This section about the uses and publication of LegacyNet data, has been substantially extended and renamed to encompass publication policies.
Version 2 to 3	Section 1.2	Additional information on the quality measurements for when the follow-on crop is a cereal has been added.
	Section 2.2	A note has been added about cultivars of species.
	Section 2.4	The text on implementing a completely randomised design has been clarified. The details of the 'Plot layout' template have been improved.
	Section 2.5.2	Table 3 about harvesting and sorting schedules now replaces the old Figure 2 (which has been removed). The range of scenarios covered has been expanded.
	Section 2.6	The text under '*Additional specific instructions for when the follow-on crop is a cereal*' has been heavily modified for clarification.
Version 3 to 4	Section 1.2	Mineral nitrogen soil measurements recommended.
	Section 2.5.2	Guidelines for species sorting updated for clarity.
Version 4 to 5	Section 1.2	Minor edits for clarification in the measurements.
	Section 2.5.1	Has been added for clarification (with following section numbers amended accordingly).
Version 5 to 6	Table 3	Note (*) added to caption.
Version 6 to 7	Section 2.5.4	Updated details / guidelines for sending forage quality samples to Kiel and payment details.

Version 7 to 8	Section 1.2	Reference to the selective herbicide in case of weed invasion removed from the table.
	Section 2.6	Text added on the treatment of a selective herbicide application as a special case for weed invasion.
Version 8 to 9	Section 1.2	Follow-on crop sections edited.
	Section 2.6 (edited considerably)	This section, on the follow-on crop phase, has been broken into five sub-sections. New text has been added for sampling of a cereal follow-on crop at anthesis and final harvest (Section 2.6.4). A new section placeholder has been added for a sampling protocol for a maize follow-on crop (Section 2.6.5); this section will be completed in the next draft of the protocol.
	Section 3	Text updated with new information on the LegacyNet Publications Committee.
Version 9 to 10	Section 2.6.4	This whole section on managing a cereal follow-on crop has been *substantially* revised.
	Section 3	LegacyNet data and publications has been removed. The LegacyNet publications policy is now available on the publications webpage: https://legacynet.scss.tcd.ie/publications.php
	Section renumbers	The current Section 3 was formerly Section 4. The current Section 4 was formerly Section 5.
	Section 2.6.4	Sentence removed to avoid ambiguity around the requirement to send samples from all harvests in the grassland phase to Kiel for quality analyses.
Version 10 to 11	Section 1.2	Some changes from mandatory to highly desirable / recommended for some follow-on cereal crop measurements.
	Section 2.6.4	Further clarifications have been made to this section and some variables recorded have changed from mandatory to highly desirable / recommended.
Version 11 to 12	Section 2.5.4	Update on charges associated with for Kiel forage quality analyses.
	Section 2.6.5	Protocol for harvesting and sampling a maize follow-on crop added.
Version 12 to 13	Section 2.6.4	Some variable names have been added and changed to make this section consistent with the updated data recording template for cereals. A minor revision has been made on how to record measurements following the removal of grain from the ears.
	Section 2.6.5	Some variable names have been added and changed to make this section consistent with the updated data recording template for maize.
Version 12 to 13	Section 2.6.4.4	Clarification of the measurements that are to be recorded on a follow-on crop cereal and names in the data recording template.

1. Introduction

1.1. Summary of the LegacyNet project

The LegacyNet project aims to implement a common experiment across multiple sites, and collect data to allow general comparisons of the effects of multi-species grassland mixtures in a crop rotation system. LegacyNet investigates the use of multi-species grassland leys as a practical farm management strategy to improve:

i) the quantity and quality of forage yield,
ii) the resource use efficiency and ecosystem services from grassland leys and
iii) the legacy effects of such grassland leys within crop rotation systems.

We use an innovative experimental design to test a range of multi-species mixtures (1-6 species) with complementary traits (grasses, legumes and herbs), and aim to design species' combinations (and proportions) that optimize forage production and legacy effects. The design of the LegacyNet experiment at each site can be split into two main parts: in the first stage, grassland plots will be established that systematically vary the diversity of six species at sowing. The plots will be sown, fertiliser applied, and harvested by mowing for a period of not less than 18 months. After at least 18 months, the second stage will commence: the grassland plots will typically be treated with herbicide and a follow-on model crop established and harvested from each individual plot over a single growing season. The follow-on crop could be a cereal, or a grassland monoculture, for example.

Relatively few experiments have been conducted that examine the impact of grassland leys in crop rotations with comparable methods across several countries, thus enabling extrapolation of benefits to different scenarios and regions. We will evaluate diverse mixtures not only for their productivity, but also their forage quality and wider ecosystem service delivery potential, as well as for the benefits that are transferred as a legacy effect into subsequent crops.

1.2. Overview of measurements

This section provides an overview of measurements. Additional experimental details are in Section 2.

Measurement	**Scale**	**Required/ Desirable?**
Field Scale		
For the duration of the experiment, daily air temperature (min, mean and max) and precipitation, either from research station where experiment located or from nearby weather station.	Field scale	Required
Soil type (usually known already for the research institute).	Field scale	Required
Soil pH	Field scale	Required
If possible, soil temperature and soil moisture (e.g., from a local weather station, not expected at a plot level).	Field scale	Desirable
If possible, C:N ratio of soil.	Field scale	Desirable
If possible, mineral nitrogen (Nmin) in soil before experiment is established (or before the first fertiliser application). Sampling from the top 10cm is recommended.	Field scale	Desirable

Stage 1: Grassland **(Please see Sections 2.5.1 – 2.5.4 for further details)**		
Biomass of grassland yield (dry matter yield) for each harvest.	Each plot and every harvest	Required
Take subsample of each harvest for forage quality analyses (see further details on NIRS in Section 2.5.3).	Each plot and every harvest	Required
Take a visual assessment of the botanical composition immediately prior to each harvest (details in Section 2.5.1).	Each plot and every harvest	Required
Determine botanical composition of all plots (2-3 times, details in section 2.5.2). This can be done, for example, by grab sampling or by the quadrat method.	Each plot (not every harvest)	Required
Mineral nitrogen (Nmin) in soil before the destruction of the grassland ley. Sampling from the top 10cm is recommended.	Each plot (prior to ley destruction)	(Highly) desirable
Termination of the grassland ley phase		
To prevent distortion of the legacy effect by clover growth in the follow-on crop, all plots are sprayed with a selective herbicide for dicots to enable clean follow-on crop establishment. If the use of herbicides (i.e. in case of organic management) is forbidden, it is allowed to terminate without herbicide use.	Each plot	Required
Stage 2: Follow-on crop, if follow-on crop is a GRASS MONOCULTURE **(Please see Sections 2.6.2 and 2.6.3 for further details)**		
Biomass of grassland yield (dry matter yield) for each harvest.	Each plot and each harvest	Required
Measure nitrogen concentration to calculate the nitrogen yield for each harvest.	Each plot and each harvest	Required
Stage 2: Follow-on crop, if follow-on crop is a CEREAL **(Please see Sections 2.6.2 and 2.6.4 for further details)** *When a cereal follow-on crop is used, the protocol is more complex than when a grass monoculture follow-on crop is used. The full protocol is available in Section 2.6.4, please read this in detail before deciding to establish a cereal follow-on crop.*		
Nitrogen analysis at full maturity of grain from whole plot, as well as grain, stems and leaves from subplot, using the Dumas method (typically analysed with Elemental Analyser). Please read Section 2.6.4 in detail for what is required.	Each plot	Required
Nitrogen analysis at anthesis of leaves and stems from subplot. Please read Section 2.6.4 in detail for what is required.	Each plot	(Highly) desirable
Stage 2: Follow-on crop, if follow-on crop is MAIZE ***(Please see Sections 2.6.2 and 2.6.5 for further details)***		
Measure fresh weight yield and determine the DM weight.	Each plot	Required
Total N yield per unit area at final harvest	Each plot	Required

2. Step-by-step instructions to establish and maintain the experiment

2.1. Field selection

Avoid any fields with a known history of problems with weeds.

The field needs to be large enough to accommodate at least 52 plots with a minimum plot size of 3m x 5m (where the follow-on crop is grass) and minimum 3m x 7m (where the follow-on crop is a cereal or arable crop). It is recommended to have pathways between the plots where possible, to reduce invasion from species in other plots. Figure 1 shows the expected plot and pathway dimensions, when the follow-on crop is a cereal. **NB: If your plot and harvested strip dimensions differ from Figure 1, please discuss with the LegacyNet Directors well in advance of establishment of your experiment.**

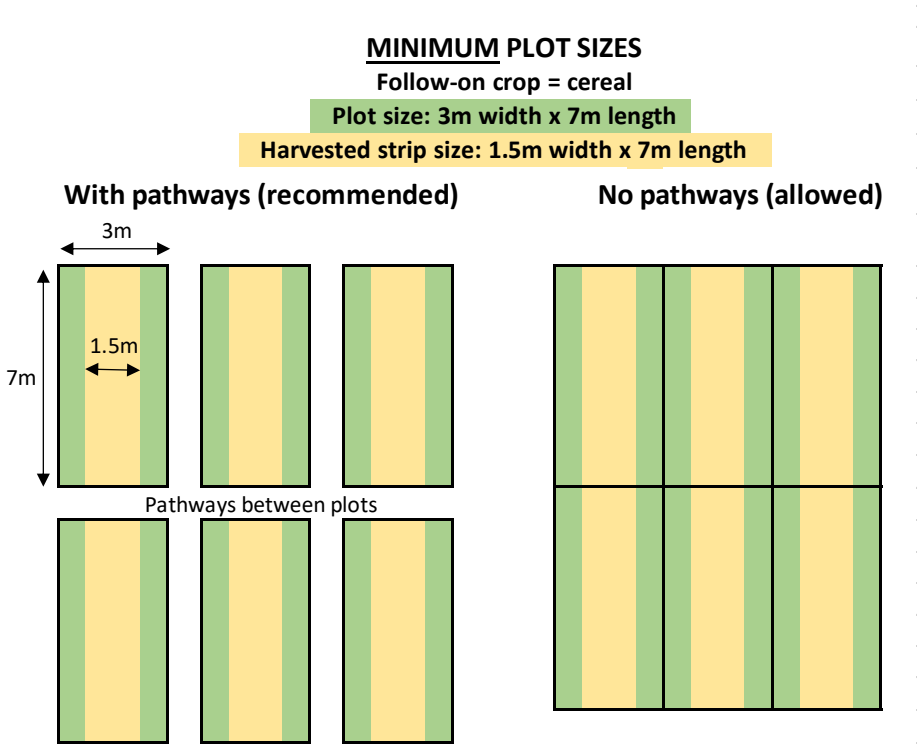

Figure 1: Illustration of the dimensions of minimum plots sizes for when the follow-on crop is a cereal. The 0.75m strip on each side of the harvested strip is part of the seeded plot, but is not used for experimental measurements; this is intended to reduce edge effects. The 7m length can be reduced to 5m in the case of a grass monoculture follow-on crop. The pathways can be anything from 40 to 100cm.

2.2. Species

Select six species that can be classified into two grasses (G1, G2), two legumes (L1, L2) and two herbs (H1, H2) and that are suitable for your regional climatic conditions. In most situations in temperate climates, the following selection of species is recommended.

G1: Perennial ryegrass - *Lolium perenne (variety)*
G2: Timothy - *Phleum pratense (variety)*
L1: White clover - *Trifolium repens (variety)*
L2: Red clover - *Trifolium pratense (variety)*
H1: Chicory - *Cichorium intybus (variety)*
H2: Ribwort plantain - *Plantago lanceolate (variety)*

However, this exact set of species does not have to be used; sets of species that are being used by individual sites are available at: https://legacynet.scss.tcd.ie/sites.php. Different cultivars of the same species can be mixed, if species proportions are not affected by this (e.g. if you mix two cultivars, use half of the recommended sowing density for each). Keep a record of your variety / cultivar(s).

2.3. Experimental design

Table 1 (next page) gives the design of the 52 core plots to be sown in the experiment.

Metadata for Table 1

- Plot = Plot number, **must be randomly distributed across the plots within a site**
- Community = unique identifier for each community with the same sown relative abundances of the six species
- NFert = low or high level of nitrogen fertiliser. Plots 1 to 47 in Table 1 will be at one level of nitrogen fertiliser addition (see Section 2.5 for guidelines). We also require a high level of nitrogen fertiliser (see Section 2.5 for guidelines) applied to additional plots of G1 only (plots 48 to 52 in Table 1). Sites with organic status can achieve the latter requirement for a high nitrogen level of G1 through application of a known additional amount of organic nitrogen.
- RichnessFG = Functional group richness
- RichnessSP = Species richness
- RichnessCAT = Categorisation of species richness: Reg for <= 6, High for >6 species.
- FG1 = G1+G2 = the relative abundance of the grass functional group at sowing
- FG2 = L1+L2 = the relative abundance of the legume functional group at sowing
- FG3 = H1+H2 = the relative abundance of the herb functional group at sowing
- G1, G2, L1, L2, H1, H2 = the relative abundance of each of the two grass, two legume and two herb species at sowing, respectively. G1 is intended to be the grass that would be chosen in monoculture at your site (e.g. *Lolium perenne* in northwest Europe). A value of 1 indicates the conventional monoculture sowing rate for that species e.g., seed weight per ha. This will differ across species and sites.

Table 1: Experimental design (metadata below). Plots 1 to 52 are the required core design – all sites must include plots 1-52 as a minimum. Each proportion is based on the seeding rate in monoculture for a species with proportion = 1. (Seeding rates will differ across species and sites. A 'seed weight calculator' assistant file is available at https://legacynet.scss.tcd.ie/experiment_details.php. Further details in Section 2.4.)

Plot	Community	Nfert	RichnessFG	RichnessSP	RichnessCAT	FG1	FG2	FG3	G1	G2	L1	L2	H1	H2
1	1	Low	1	1	Reg	1	0	0	1	0	0	0	0	0
2	1	Low	1	1	Reg	1	0	0	1	0	0	0	0	0
3	1	Low	1	1	Reg	1	0	0	1	0	0	0	0	0
4	2	Low	1	1	Reg	1	0	0	0	1	0	0	0	0
5	2	Low	1	1	Reg	1	0	0	0	1	0	0	0	0
6	2	Low	1	1	Reg	1	0	0	0	1	0	0	0	0
7	3	Low	1	1	Reg	0	1	0	0	0	1	0	0	0
8	3	Low	1	1	Reg	0	1	0	0	0	1	0	0	0
9	3	Low	1	1	Reg	0	1	0	0	0	1	0	0	0
10	4	Low	1	1	Reg	0	1	0	0	0	0	1	0	0
11	4	Low	1	1	Reg	0	1	0	0	0	0	1	0	0
12	4	Low	1	1	Reg	0	1	0	0	0	0	1	0	0
13	5	Low	1	1	Reg	0	0	1	0	0	0	0	1	0
14	5	Low	1	1	Reg	0	0	1	0	0	0	0	1	0
15	5	Low	1	1	Reg	0	0	1	0	0	0	0	1	0
16	6	Low	1	1	Reg	0	0	1	0	0	0	0	0	1
17	6	Low	1	1	Reg	0	0	1	0	0	0	0	0	1
18	6	Low	1	1	Reg	0	0	1	0	0	0	0	0	1
19	7	Low	1	2	Reg	1	0	0	0.5	0.5	0	0	0	0
20	8	Low	1	2	Reg	0	1	0	0	0	0.5	0.5	0	0
21	9	Low	1	2	Reg	0	0	1	0	0	0	0	0.5	0.5
22	10	Low	2	2	Reg	0.5	0.5	0	0.5	0	0.5	0	0	0
23	11	Low	2	2	Reg	0.5	0.5	0	0.5	0	0	0.5	0	0
24	12	Low	2	2	Reg	0.5	0	0.5	0.5	0	0	0	0.5	0
25	13	Low	2	2	Reg	0.5	0	0.5	0.5	0	0	0	0	0.5
26	14	Low	2	2	Reg	0.5	0.5	0	0	0.5	0.5	0	0	0
27	15	Low	2	2	Reg	0.5	0.5	0	0	0.5	0	0.5	0	0
28	16	Low	2	2	Reg	0.5	0	0.5	0	0.5	0	0	0.5	0
29	17	Low	2	2	Reg	0.5	0	0.5	0	0.5	0	0	0	0.5
30	18	Low	2	2	Reg	0	0.5	0.5	0	0	0.5	0	0.5	0
31	19	Low	2	2	Reg	0	0.5	0.5	0	0	0.5	0	0	0.5
32	20	Low	2	2	Reg	0	0.5	0.5	0	0	0	0.5	0.5	0
33	21	Low	2	2	Reg	0	0.5	0.5	0	0	0	0.5	0	0.5
34	22	Low	3	3	Reg	0.33	0.33	0.33	0.33	0	0.33	0	0.33	0
35	23	Low	3	3	Reg	0.33	0.33	0.33	0.33	0	0.33	0	0	0.33
36	24	Low	3	3	Reg	0.33	0.33	0.33	0.33	0	0	0.33	0.33	0
37	25	Low	3	3	Reg	0.33	0.33	0.33	0.33	0	0	0.33	0	0.33
38	26	Low	3	3	Reg	0.33	0.33	0.33	0	0.33	0.33	0	0.33	0
39	27	Low	3	3	Reg	0.33	0.33	0.33	0	0.33	0.33	0	0	0.33
40	28	Low	3	3	Reg	0.33	0.33	0.33	0	0.33	0	0.33	0.33	0
41	29	Low	3	3	Reg	0.33	0.33	0.33	0	0.33	0	0.33	0	0.33
42	30	Low	2	4	Reg	0.5	0.5	0	0.25	0.25	0.25	0.25	0	0
43	31	Low	2	4	Reg	0.5	0	0.5	0.25	0.25	0	0	0.25	0.25
44	32	Low	2	4	Reg	0	0.5	0.5	0	0	0.25	0.25	0.25	0.25
45	33	Low	3	6	Reg	0.33	0.33	0.33	0.17	0.17	0.17	0.17	0.17	0.17
46	33	Low	3	6	Reg	0.33	0.33	0.33	0.17	0.17	0.17	0.17	0.17	0.17
47	33	Low	3	6	Reg	0.33	0.33	0.33	0.17	0.17	0.17	0.17	0.17	0.17
48	34	High	1	1	Reg	1	0	0	1	0	0	0	0	0
49	34	High	1	1	Reg	1	0	0	1	0	0	0	0	0
50	34	High	1	1	Reg	1	0	0	1	0	0	0	0	0
51	34	High	1	1	Reg	1	0	0	1	0	0	0	0	0
52	34	High	1	1	Reg	1	0	0	1	0	0	0	0	0
53	35	Low	3		High	These five plots are optional, but recommended. If you choose to sow								
54	35	Low	3		High	these plots, please use as high a diversity mix of seed as possible. All six								
55	35	Low	3		High	regular experimental species should be chosen, plus as many more as								
56	35	Low	3		High	possible, e.g. 12 species in total, with four from each functional group,								
57	35	Low	3		High	to include the original six species.								

2.3.1. Additional treatments

In Table 1, plots 1 to 52 are the required core design – all sites must include plots 1-52 as a minimum.

Plots 53 to 57 represent an optional treatment that is recommended, and similar measurements should be taken from these plots. Plots 53 to 57 should be sown with as high diversity as possible, and this may reflect a locally available seed mix for grazed pastures. As an example only, if your six species in the core design are: perennial ryegrass, timothy, white clover, red clover, chicory, ribwort plantain, then plots 53-57 could be a mix of perennial ryegrass, timothy, cocksfoot, Festuca sp., red clover, white clover, birds foot trefoil, vetch, plantain, chicory, yarrow, salad burnet (12 species, suited to temperate grasslands). Alternatively, you could simply use a locally available high-diversity seed mixture that is available for purchase. **You would not be required to do any manual separation of these high diversity plots.** The underlying question addressed here is whether the performance of the six-species mixture is any better than sowing a higher number of species in a mixture that is allowed to develop and lets the 'best' species win.

Individual sites are also welcome (and encouraged!) to increase the number of plots they sow to test an additional treatment.

If other additional treatments or measurements are of interest to you (great!), please discuss your plans with the LegacyNet Directors in advance of establishing your experiment. We can then assist with design or coordination in case any other sites have a similar idea.

Some ideas for additional treatments / measurements:

- Sow double the number of plots and use two fertiliser levels.
- If just small amounts of additional plots are available, sow some additional "optimal" follow-on crops, where both in the grass and follow-on crop, a cereal or ryegrass is cultivated under best practice conditions, with sufficient fertiliser application, herbicides etc. This is the resource-intensive benchmark that the other plots compete against, grown under identical environmental conditions. The list of benchmarks can of course be expanded indefinitely, including different fertiliser levels, legumes undersown, different establishment techniques and so forth.
- Determine Nitrogen uptake by plants using tracers (recommended for groups that are interested in using 15N). Regardless of whether the follow-on crop is a grass or a cereal, 15N with an isotopic purity of > 60 atom % should be added together with the **first** fertilisation (in case of a grass follow-on crop) or **only** fertilisation (in the case a cereal follow-on crop). **Please indicate your willingness to implement 15N studies to the LegacyNet Directors, so that a detailed common protocol can be developed to ensure uniformity across sites.**

To be clear, no site is required to do any additional plots on top of the core 52 plots, or to take additional measurements. However, this may give sites a unique site-specific option for publishing. Or if multiple groups do the same additional treatment or measurements, there is an opportunity for a joint publication on this work for just those sites, with statistical support provided.

2.4. Sowing

An Excel template file has been provided (at https://legacynet.scss.tcd.ie/experiment_details.php) to calculate the amount of seeds required for each individual plot based on the thousand kernel weight and the seeding rate for each species (for examples of seeding rates in other partner countries, see Table 2).

Table 2. Comparison of seeding rates and nitrogen application in some of the current participating countries, kg ha^{-1}. Thus, these seeding rates correspond to proportion = 1 in Table 1.

	Nitrogen rates	Perennial ryegrass	Timothy	White clover	Red clover	Chicory	Ribwort plantain
Ireland	low 150 high 300	30 (50:50 diploid and tetraploid)	12	15 (coated)	12	8	10
Germany		30 (50:50 diploid and tetraploid)	15	5	12	18	12
Canada	low 100 high 300	28	12	10	12	10	10

Implement a **completely randomised design**, i.e., the 52 (or 57 if you include the high diversity treatment) plots in Table 1 should randomly assigned to the plots in your field. Should you need assistance, please contact the LegacyNet Directors.

Prior to establishing, prepare a visual layout of your field showing your randomisation schedule, the dimensions of your plots and the dimensions of the part of the plot that will be harvested for experimental measurements, relative to plot width. **Use the 'Plot layout' template provided at** https://legacynet.scss.tcd.ie/experiment_details.php**.** Please also give your harvesting method, where requested in the file. **THIS FILE MUST BE SENT TO THE LEGACYNET DIRECTORS PRIOR TO ESTABLISHING YOUR EXPERIMENT.**

Prepare a stale seedbed using shallow cultivation by harrowing to reduce the weed pressure in the field, and a second shallow cultivation approximately 12 days later, followed by seeding.

Sow the experiment using either manual sowing or a machine for sowing seed. Use a roller after sowing to ensure soil-seed contact.

If required (if no legumes were used at the site for a long time), inoculate legumes with Rhizobia. P and K fertiliser should be applied according to national guidelines and soil nutrient status in order to not be limiting. P and K should be applied either before seeding or immediately after germination.

2.5. Stage 1: The grass growth stage

No weeding (either hand, mechanical or chemical) should be conducted during the experiment. The only exception in case of a prevalence of annual weeds after establishment is a cleaning cut. A cleaning cut is a harvest performed within 1-6 months of establishment depending on the timing of establishment (and the climate of the site) to promote the growth of the sown species over annual weeds. **If you have a concern about establishment of the experiment due to a high volume of weeds, please contact the LegacyNet Directors.** For example, if there is >20% weeds in 10 or more of the plots in the first visual survey after establishment and after a cleaning cut, then the experiment may need to be re-established.

Two levels of nitrogen should be applied, low and high. The high level of nitrogen on selected plots is to estimate the extent to which the different mixtures can replace nitrogen/ attain a high level of yield for a site.

- A low N treatment (e.g. 50 - 150 kg N ha^{-1}) must be applied to Plot 1-47 in Table 1.
- A high N treatment (e.g. 150 - 450 kg N ha^{-1}) must be applied to Plot 48-52 in Table 1.

The chosen levels of nitrogen addition may vary substantially across global sites, reflecting their local productivity (the low level may even be zero at some sites). However, please ensure that there is at least a difference of 100 kg N ha^{-1} between the low and high level.

Nitrogen can be applied as either mineral fertilizer or organic nitrogen (the latter especially for sites with organic status). Nitrogen as mineral fertilizer should be applied in spring, as well as after each cut (with the possible exception in autumn) with the highest application in spring.
Harvest according to regional management practices (with range from 2-7 harvests per year) to determine the biomass. Harvest should occur using a mechanical harvester to get representative wet weights per plot. If you do not plan to use a mechanical harvester, please discuss your planned method with the LegacyNet Directors. Picture of a mechanical harvester at work:

2.5.1. Dry matter yield

It is expected that LegacyNet participants have experience with plot-scale grassland trials and have established methods for reliably computing dry matter yields for plot-scale harvests. Please contact us if you need assistance with this. Note that dry matter yields should be directly measured by harvesting and not estimated (using for example plate meter methods).

2.5.2. Visual assessment of species proportions

Immediately before **each harvest**, take a visual assessment of the species proportions in each plot. This visual assessment is meant to approximate the biomass that is present for each species in the plot. It is understood that this is an approximation and not totally accurate, but it will provide an important signal as to what the species proportions are and will show if some species become particularly dominant, or if some species disappear altogether.

2.5.3. Sorting botanical composition

Select two to three harvests to sort for botanic composition; the specific harvests and the number of harvests you choose to sort will depend on how many harvests you take, recommendations in Table 3. The number of harvests per year is determined by local practice. If you have the resources to manually separate every harvest, you can, but this is not required.

Table 3: LegacyNet harvesting and sorting schedule for varying number of harvests and spring / autumn sowing of the follow-on crop. Establishment can also be in 2021. *Note: in (a), if you have concerns about the feasibility of the harvest of the grassland ley in Spring of the second year (before the crop establishment), please contact the LegacyNet Directors.

(a) For a spring sowing follow-on crop*			
# harvests	**2020**	**2021**	**2022**
3	Sow in Autumn	H1, H2, H3 **Required sorting**: H2 Recommended sorting: H2, H3	H1 **Required sorting**: H1 Sow follow-on crop in Spring
4	Sow in Autumn	H1, H2, H3, H4 **Required sorting**: H3 Recommended sorting: H2, H3	H1 **Required sorting**: H1 Sow follow-on crop in Spring
5	Sow in Autumn	H1, H2, H3, H4, H5 **Required sorting**: H4 Recommended sorting: H2, H4	H1 **Required sorting**: H1 Sow follow-on crop in Spring
6	Sow in Autumn	H1, H2, H3, H4, H5, H6 **Required sorting**: H5 Recommended sorting: H2, H5	H1 **Required sorting**: H1 Sow follow-on crop in Spring
7	Sow in Autumn	H1, H2, H3, H4, H5, H6, H7 **Required sorting**: H6 Recommended sorting: H2, H6	H1 **Required sorting**: H1 Sow follow-on crop in Spring
(b) For an autumn sowing follow-on crop			
# harvests	**2020**	**2021**	**2022**
3	Sow in Autumn	H1, H2, H3 **Required sorting**: H2 Recommended sorting: H2, H3	H1, H2, H3 **Required sorting**: H2, H3 Sow follow-on crop in Autumn
4	Sow in Autumn	H1, H2, H3, H4 **Required sorting**: H3 Recommended sorting: H2, H3	H1, H2, H3, H4 **Required sorting**: H2, H4 Sow follow-on crop in Autumn
5	Sow in Autumn	H1, H2, H3, H4, H5 **Required sorting**: H4 Recommended sorting: H2, H4	H1, H2, H3, H4, H5 **Required sorting**: H2, H5 Sow follow-on crop in Autumn
6	Sow in Autumn	H1, H2, H3, H4, H5, H6 **Required sorting**: H5 Recommended sorting: H2, H5	H1, H2, H3, H4, H5, H6 **Required sorting**: H2, H6 Sow follow-on crop in Autumn
7	Sow in Autumn	H1, H2, H3, H4, H5, H6, H7 **Required sorting**: H6 Recommended sorting: H2, H6	H1, H2, H3, H4, H5, H6, H7 **Required sorting**: H2, H7 Sow follow-on crop in Autumn

Guidelines for sorting

- Harvest to a height of 5 cm
- Subsample plots either using a quadrat of 50 cm x 50 cm or by grabbing 10 random subsamples. If using grab sampling, a minimum 350g wet weight is required per grab sample for a single plot.
- Split the sample into two parts: One of which is used to determine the dry matter concentration in the sample to determine the dry matter yields of the whole sward, while the other part is used for fractionation
- Sort the part for botanic compositions into each of the species that were sown in the plot and weeds. Weed species are only reported as a group, and do not need to be identified to species level. Note that species that are in the experimental pool, but were not sown in the plot should be included in weeds. For example, if sorting biomass from a monoculture of G1,

if any of G2, L1, L2, H1 or H2 are found in the plot sample, they should be included in the weeds as they were not sown in the plot.
- Dry the samples per species to constant weight and weigh to determining dry weight.
- Convert the biomass for each species into a proportion before entering into the data recording template.

2.5.4. Determining forage quality

Take a subsample **from each plot and each harvest** to determine forage quality to be analysed at CAU Kiel. There is no longer a cost for the analyses (as of 2023). We need the analyses of these samples to be performed at a single laboratory, for consistency across these scientific measurements. NIRS analyses carried out at Kiel will provide estimates for the following parameters: nitrogen content, carbon content, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash content, fat and in vitro pepsin cellulase solubility of organic matter according to de Boever (CDOMD).

Guidelines for subsampling for NIRS analyses

- Take representative subsample (ca. 50-100 g wet weight) from the harvested material
- Oven dry material at 60°C until constant weight
- Mill sample in knife mill using a sieve size of 1 mm
- Homogenize the milled material and take subsample of at least 2 g DM (better 4-5 g) in a vial to be shipped for analyses.
- Label each vial with the following convention:
    - Site ID . Stage . HarvestN . Plot .
        - Where Site ID is your unique site identifier number as listed on the LegacyNet website (https://legacynet.scss.tcd.ie/sites.php);
        - Stage is either 'G' for Grass or 'F' for Follow-on crop depending on if the sample is from Stage 1 the **G**rassland phase, or Stage 2 the **F**ollow-on crop stage;
        - HarvestN is the number of the harvest;
        - Plot is a number ranging from 1 to 52 and matches with the corresponding set of species proportions as laid out in Table 1.

      HarvestN and Plot must match with the data recording spreadsheet (available at https://legacynet.scss.tcd.ie/experiment_details.php).
      As an example, the sample from the second harvest in Wexford, Ireland with a complex mixture (6 species in equal proportions) in the grassland stage would be labelled as **IE1.G.2.45** where IE1 is the unique identifier for Wexford, G indicates the grassland stage 1, 2 indicates the second harvest and 45 is the plot number of the first rep of the six-species equi-proportional mixture (see Table 1).
    - If you have additional plots beyond the core 52, make sure to number them higher than 52 in the Plot column in the data recording spreadsheet and on the vial.
    - If is imperative that care is taken with the labelling convention so that each vial can be uniquely matched to a row in your data recording spreadsheet.
- Make sure the label does not rub off under friction as it frequently occurs during transport.
- Store safely and send to:

Petra Voss
Grünland und Futterbau / Ökol. Landbau
Hermann-Rodewald Str. 9
24118 Kiel
Germany

To make sure that the organization runs smoothly, please send an email containing your sample list (and the sown botanic composition from each sample as indicated in Table 1 of this protocol) parallel to shipping the samples to pvoss@gfo.uni-kiel.de with cmalisch@agro.au.dk in cc, so that we have an overview of whose samples have been submitted for analysis.

2.6. Stage 2: The follow-on crop stage

At your site, you may use a grass monoculture, a cereal, or a maize follow-on crop. In this section, we detail first how to terminate the grassland ley phase and then how to manage the various types of follow-on crop.

2.6.1. Terminating the grassland ley phase

After a minimum of 18 months after establishment: Terminate grassland leys, and sow the follow-on crop. **IMPORTANT: Maintain the same plot structure for this stage.** To measure the legacy effect, we will need to harvest the follow-on crop from the same plot area as for the ley. Thus, you need to mark the site dimensions and layout to be able to impose and harvest the follow-on crop from the same plot locations as where the grassland plots were located.

Terminate the ley using one of the following two options:

- **Option A (preferred)**: use a herbicide to kill the ley vegetation. Dependent on regional conditions, a one-month waiting period between the ley dying off and the sowing of the cereal might be required to reduce fruit-fly risks. Use either a disc harrow or a rotary hoe as minimal tillage to convert to follow-on crop (either cereal or annual grass such as Italian ryegrass (*Lolium multiflorum*)). Sow seeds into the seedbed. In case of cereal, use a drill for sowing. **DO NOT PLOUGH.**
- **Option B (NB: for organic systems only)**: Instead of using a herbicide, use a disc harrow twice (at the beginning and end of a 10-day period) to destroy the grassland leys, before ploughing to a depth no deeper than 30 cm. Sow seeds into the seedbed.

2.6.2. Establishment and management of the follow-on crop: in general

Use a sowing density of annual ryegrass or cereal according to local recommendations. If unsure, contact a local agricultural advisor or the LegacyNet Directors.

Provide P and K according to soil nutrient status and regional recommendations, either before sowing or after germination.

Minimal amounts of nitrogen fertiliser or organic nitrogen should only be applied to the follow-on crop. Specifically, **no fertiliser should be added in the establishment** to avoid dilution of the ley effects. (If you feel your site requires fertiliser addition at this stage, please check the soil Nmin levels and contact the LegacyNet Directors to discuss). After establishment, locally adapted low N amounts of fertiliser should be used.

- In the case of a grass follow-on crop, no more than 30 kg N ha^{-1} per cut and in the case of only one cut, no fertiliser at all should be added.
- In case of a cereal crop, fertiliser application should occur as late as possible (around the tillering stage) and also with minimal (locally adapted) fertilisation levels (not more than **50** kg N ha^{-1} for a cereal follow-on crop).

2.6.3. Protocol for harvesting and sampling of GRASS follow-on crop

Harvest the grassland for at least six months during the growing season after sowing. For less productive sites, this may mean that there are two harvests; for more productive sites, there may be four or more harvests in this time; it is possible for there to be only one harvest, but we expect this to be the case for a very small number of sites.

Take a subsample **from each plot and each harvest** to determine forage quality to be analysed at CAU Kiel for a cost of €5 **per sample (at your own cost)**. See guidelines above (Section 2.5.4) for subsampling for NIRS analyses. Follow the labelling convention in 2.5.4 to label your samples and ensure to indicate that Stage = F for the follow-on samples.

2.6.4. Protocol for harvesting and sampling of CEREAL follow-on crop at anthesis and final harvest

2.6.4.1. Overview of cereal harvesting and sampling

There are two important measurement / sampling periods for a cereal follow-on crop:

1) **Highly recommended**: At anthesis, measurements should be taken locally, *and* samples should be taken from each plot and analysed for nitrogen concentration using the Dumas method.
2) **Required**: At maturity of the cereal (the final harvest), measurements need to be taken and recorded on each plot locally, *and* samples need to be taken from each plot and analysed for nitrogen concentration using the Dumas method.

The measures will no longer be analysed centrally, due to a high comparability of data from different laboratories if the same analytical method is used.

If you have opted to establish a cereal follow-on crop, but you are not experienced in working with cereals, we *strongly recommend* that you read this part of the protocol in detail well in advance of establishing your follow-on crop. Please also talk to the LegacyNet Directors who can put you in touch with colleagues who have already implemented this part of the protocol for advice.

2.6.4.2. How to determine when anthesis and maturity are reached

When has my cereal crop reached the anthesis stage?

The intention is that no plots will have passed BBCH stage 69 and the majority or all plots will have attained at least BBCH 65. To determine the date of anthesis sampling, we recommend:

- In the lead up to the expected anthesis time, use visual assessment of the stage of cereal maturity to guide the anthesis date.
- Once anthesis is observed at multiple plots (anthesis defined here as BBCH stage 65, full flowering: 50% of anthers mature), sample all of the plots and record the individual BBCH stage for each plot to identify variability).
- The intention is that no plots will have passed BBCH stage 69 and the majority or all plots will have attained at least BBCH 65. Individual sites may require fewer or more days.
- It is crucial that all plots are measured and sampled on the same day.

When has my cereal crop reached the maturity stage?

- Visually assess and aim to do the final harvest of your cereal crop at maturity, defined as BBCH stage 89-92 and dead ripeness.
- It is crucial that all plots are sampled and harvested on the same day.

2.6.4.3. Overview of how to measure cereal crops

At anthesis, measurements will initially be recorded on 10 plants OR 20 tillers (e.g. see example with black dots in brown border area in Fig. 2), and, after that, on two rows of plants (e.g. blue bars in brown border area in Fig. 2). At maturity, measurements will initially be recorded on two rows (e.g. grey bars in yellow interior area) and, after that, on the full mechanically harvested (full interior yellow area).

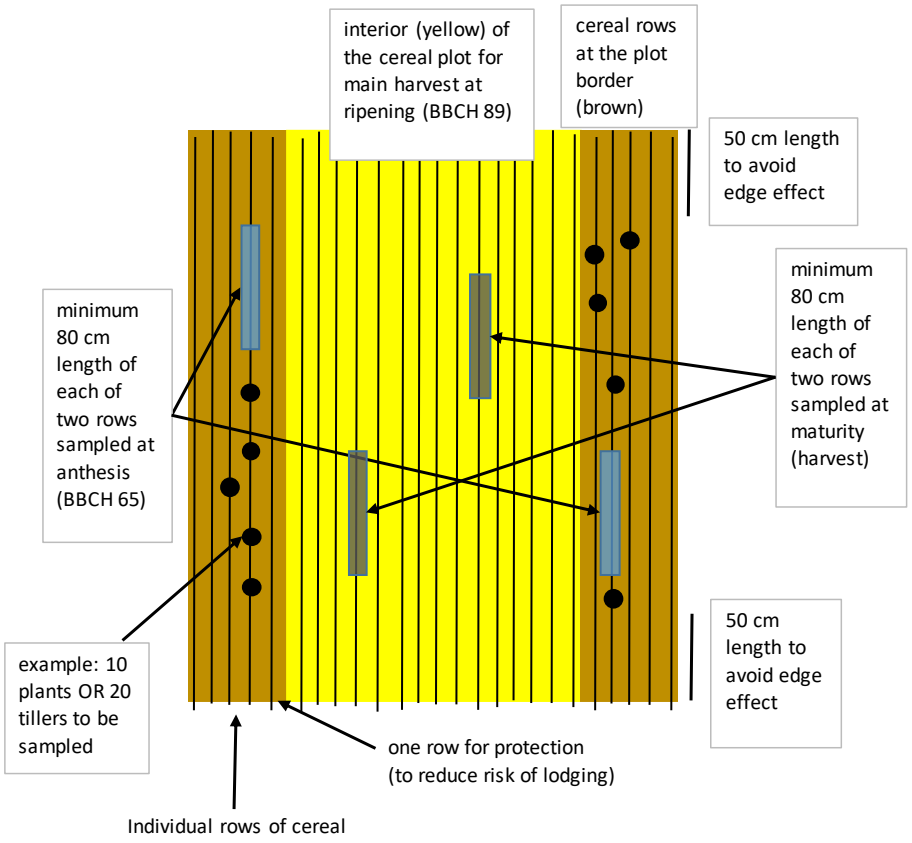

Figure 2. Representation of the cereal plot, and indicative location of the sampling at anthesis (light blue box to estimate N yield at anthesis) and at maturity (dark grey box). Internal yellow area is the main area to be harvested at maturity for most representative sample of grain yield. Border (brown areas) are to be sampled at anthesis to reduce disturbance and risk of lodging the crop for harvest at maturity. Black dots indicate examples of the arbitrary locations of sampling of 10 plants (for N fractionation) in the border area only. (Sampling for 20 tillers not shown.)

The reason for taking nitrogen concentrations measurements (details later) at two points is that comparing these data to soil Nmin concentrations will allow us to calculate the nitrogen uptake efficiency, while the differences in N concentrations across fractions from anthesis to harvest allows one to trace N pathways within the plant and calculate the crop N Remobilization Efficiency. With these methods, we can determine a) how much of the soil nitrogen is being used by the plant, and how much ends up in the harvestable product, as well as the total nitrogen yield. **The analyses at anthesis are not mandatory, but are required to participate in the anticipated specific paper on the legacy effect on N relocation and pathways in cereal crops. Total nitrogen yield per unit area is a**

common variable that links the legacy effect in the all-site combined analysis across different crop types and across all sites and is therefore a required measure.

2.6.4.4. Summary of CEREAL samples for analyses

For the whole cereal N analyses, that cover all measures for the anticipated cereal multisite publications, a total of 9 samples is required. The minimum requirement that is non-optional are 5 samples (samples 5-9 in list below)

AT ANTHESIS **(highly desirable, and required for N relocation publication)**:

From **at least** 10 plants OR 20 tillers from different parts of the border area (make sure sample is sufficiently large to take a representative subsample (to create a minimum of 5g dry weight per subsample)):

1. subsample from the **ears**, dry at 60°C, milled to 2 mm
2. subsample from the **stems**, dry at 60°C, milled to 2 mm
3. subsample from the **leaves**, dry at 60°C, milled to 2 mm

From the **2 rows** of 80 cm in length in the border area:

4. subsample from the **whole harvested biomass**, dry at 60°C, milled to 2 mm

AT HARVEST **(required)**:

From the **2 rows** of 80 cm in length within the main harvesting area:

5. subsample from the **grains,** dried at 30°C, milled to 2 mm
6. subsample from the **stems**, dried at 60°C, milled to 2 mm
7. subsample from the **leaves**, dried at 60°C, milled to 2 mm

Mechanically harvested area of the main harvesting area:

Subsample **grains** from the full harvested area, dry at 30°C and divide this sample at random into:

8. subsample from the **grains,** >100 g, (do not mill), and:
9. subsample from the **grains,** 20 g, milled to 2 mm

Full details of how to obtain these samples, and the measurements that need to be recorded locally at the site are indicated below.

The following table describes the measurements to be recorded and sampling methods to be used, and indicates the respective column in the recording spreadsheet where the results should be listed; the table is split into the anthesis and harvest stages.

AT PLANTING	
Information on methods	**Required or recommended**
Record the row width that you used at planting. This will be important information for scaling some measurements later on.	Required (and very important!)
AT ANTHESIS	
Information on methods (names in brackets indicate respective column in recording spreadsheet)	**Required or recommended**
• Record BBCH stage of development for each plot at the time of anthesis measuring (Anth_BBCH)	Recommended

Methods for measuring and sampling plants or tillers at anthesis: • Select (at random) **at least** 10 plants OR 20 tillers (while noting number of harvested plants/tillers) from different parts of the border area immediately outside the main harvest area (see Fig. 2) and cut to ground level. • Make sure enough material (especially leaves) is available for analysis (to create a minimum of 5g dry weight per subsample). We focus on the border area to reduce disturbance and risk of lodging in the main harvest area. • Count and record either the number of stem fractions (Anth_Stem_Count_Fr), or the number of plant fractions (Anth_Plant_Count_Fr) (whichever you took) on each plot. • Separate into ear, stem, leaf, and combine the ear fractions, combine the stem fractions, combine the leaf fractions (across the plant samples within a single plot). The samples for each fraction will be dried in a ventilated oven at 60 degrees Celsius until constant weight, the dry matter of each fraction will be weighed, i.e. separately for Ear, Stem and Leaf (Anth_Ear_DMY_Fr, Anth_Stem_DMY_Fr, and Anth_Leaf_DMY_Fr; These are samples 1-3 in the list at the beginning of this section). • Mill (to 2 mm) the ear fraction from the multiple plants/tillers, the stem fraction from the multiple plants/tillers, the leaf fraction from the multiple plants/tillers. • Analyse these samples using the Dumas method (Anth_Ear_N_Fr_Con, Anth_Stem_N_Fr_Con, Anth_Leaf_N_Fr_Con). If you do not have access to the relevant equipment or are in doubt, please contact the LegacyNet Directors and we will assist you in finding a suitable laboratory. There will be a charge by the laboratory.	(Highly) recommended
Methods for measuring and sampling 2 rows of 80 cm in border area at anthesis: • Cut all plants at ground level in two rows to a minimum length of 80 cm each, namely each row on the edge of the main harvest area (see Fig. 2). (Knowing the row width means we can scale to a known area to calculate total N yield per unit area) There is no need to separate into fractions. • These will be dried in a ventilated oven at 60 degrees Celsius until constant weight, then the dry matter will be weighed (Anth_Comb2Rows_DMY) (This is sample 4 in the list at the beginning of this section). • Mill all the harvested material to 2 mm. • Analyse these samples using the Dumas method (Anth_Comb2Rows_N_Con). If you do not have access to the relevant equipment or are in doubt, please contact the LegacyNet Directors and we will assist you in finding a suitable laboratory. There will be a charge by the laboratory. This N response measurement deepens understanding of nitrogen dynamics at anthesis, complements total N yield at maturity and is a backup in case of any problem with the final harvest (e.g. predation of grain by pests).	(Highly) recommended
FINAL HARVEST AT CEREAL MATURITY	
Information on methods (names in brackets indicate respective column in recording spreadsheet)	**Required or recommended**
• Record BBCH stage of development for each plot at the time of maturity measuring (Mat_BBCH)	Recommended

Methods for measuring and sampling two rows prior to mechanical harvesting: • From the main harvest area of each plot, harvest 2 rows of 80 cm each to ground level Dry the harvested plants in an oven at **30 Degrees** Celsius temperature to constant weight (low temperature to avoid changes in grain N). Remove the samples from the oven and weigh them (Mat_Comb2Rows_DMY). • Cut off the ears (exactly below them, to not include any stem). **Count ears** (Mat_Ear_Count_Fr) while cutting and weigh all removed ears from each plot (Mat_Ear_DMY_Fr). Counting of ears is to determine the average N concentration in ear-bearing stems, as a safety measure in case swards are heterogenous. Alternatively, counting stems (Mat_Stem_Count_Fr) or plants (Mat_Plant_Count_Fr) is also fine. • Remove grains from ears, e.g. by threshing and winnowing (final fractions: leaves, stems and grains). There is no recommended way to do this – separation can thus vary across sites but should be consistent within each site. Weigh the grains (Mat_Grain_DMY_Fr). • Place remaining plant biomass in oven again **at 60 degrees Celsius** until constant weight • Separate the remaining plant material into leaves and stems. **Weigh** each fraction (Mat_Leaf_DMY_Fr, Mat_Stem_DMY_Fr). (Note that the Stem weight should not include the difference between the Ear and the Grain, this can be computed by the Statistics Team at a later date as required.) • Thus, three final biomass fractions will be: grains, leaves and stems. • Mill the grain, leaves and stem material (separately) to 2 mm. • Analyse these samples using the Dumas method. If you do not have access to the relevant equipment or are in doubt, please contact the board of directors and we will assist you in finding a suitable laboratory (Mat_Grain_N_Fr_Con, Mat_Leaf_N_Fr_Con, Mat_Stem_N_Fr_Con, Mat_Grain_N_Fr_Con).	Required
Methods for mechanical harvesting, measuring and sampling of the full harvesting area at maturity: • After harvesting, threshing and winnowing is required to separate grains from husks and other residues • Record grain yield (wet weight, Mat_FullHarv_Grain_WMY) of the plot from mechanical harvesting. (If you harvested before finalisation of the cereal protocol and cannot retrieve the grain wet weight, please record the dry weight of the grain yield (Mat_FullHarv_Grain_DMY) instead.) • Take a subsample of at least 200 g of the grains from each plot and record the exact weight of the subsample (Mat_FullHarv_GrainSub_WMY). Dry subsample in a ventilated oven at 30 degrees Celsius until constant weight. Measure the DM weight of the subsample and record (Mat_FullHarv_GrainSub_DMY). • Divide the dried subsample into a sample of size 100 g and a sample of size 20 g. Any remaining material can be stored as backup. • Analyse this sample (100 g) method for thousand kernel weight (Mat_TKW) and size grain distribution. Sieve sizes should give following categories (in mm) of grain size distribution: >2.8, 2.8-2.5, 2.5-2,2, <2.2 (Mat_GrainSize_2.8, Mat_GrainSize_2.8_2.5, Mat_GrainSize_2.5_2.2, Mat_GrainSize_2.2 respectively). Do NOT mill this sample. (This is sample number 8 in the list at the beginning of this section). If you don't have access to equipment to measure thousand kernel weight or grain size distribution, contact the	Required

LegacyNet board of directors and we will help you get in touch with laboratories to assist you. • Mill the remaining 20 g subsample to 2 mm (for each plot) and analyse for N concentration using Dumas method (Mat_FullHarv_Grain_N) to determine N yield (one sample per plot). (This is sample number 9 in the list at the beginning of this section). If you do not have access to the relevant equipment or are in doubt, please contact the board of directors and we will assist you in finding a suitable laboratory.	

2.6.5. Protocol for harvesting and sampling of silage maize follow-on crop

In summary:

AT FINAL HARVEST:

- Harvest the whole plot, record the fresh weight yield and determine the DM weight.
- Take a representative subsample of **whole harvested biomass** from the mechanically harvested area**,** dried at 60°C (milled to 1 mm) for analyses. This subsample should be >20 g dry weight.

In detail:

FINAL HARVEST OF SILAGE MAIZE		
	Information on methods	Required / recommended
Timing of harvest	Visually assess and aim to harvest at 36 % DM (whole plant). Harvest all plots on the same day. (This corresponds roughly to 55-60% cob DM and 24-27 % crop DM. Usually achieved when 50 – 25 % of leaves is still green.)	Required
Mechanical harvesting Total N yield per unit area at final harvest	Mechanical harvest of main harvest area in the center of the plot. • Use a plot harvester with weighing and sampling device (for example 2-row Haldrup). Alternatively, harvest and chop the plants manually (see above) • Determine and record the fresh weight yield of the plot (whole plant), and harvest area (WMY). • Take a subsample, and then weigh it (WMY_SUB) of the chopped plant from each plot, and dry in a ventilated oven at 60 degrees Celsius. Determine and record DM weight (DMY_SUB). • Test a representative subsample of the plant material (>20g) to determine N concentration (one sample per plot) (N_Conc). Analyse these samples using the Dumas method. If you do not have access to the relevant equipment or are in doubt, please contact the LegacyNet Directors and we will assist you in finding a suitable laboratory. There will be a charge by the laboratory.	Required

3. Statistical analysis

We will use the Diversity-Interactions regression analysis approach (Kirwan et al. 2009, Dooley et al. 2015) which includes the species proportions and their interactions as predictors of single ecosystem functions and of ecosystem multifunctionality (multiple responses). Using this approach, the

community-level responses will be modelled as a linear combination of i) identity effects of species to represent their monoculture performance, and ii) species net interactions, termed diversity effects (positive, negative, or neutral), which are defined as the difference between the mixture performance and that expected from the relative contribution of the constituent monocultures. Statistical expertise is available within LegacyNet to facilitate combined analysis of all-site data.

Kirwan L, J Connolly, JA Finn, C Brophy, A Lüscher, D Nyfeler and MT Sebastià (2009) Diversity-interaction modelling - estimating contributions of species identities and interactions to ecosystem function. Ecology, 90, 2032–2038.

Dooley Á, F Isbell, L Kirwan, J Connolly, JA Finn and C Brophy (2015) Testing the effects of diversity on ecosystem multifunctionality using a multivariate model. Ecology Letters 18, 1242-1251

4. Anticipated outputs from the LegacyNet common experiment

There will be several multi-site papers arising from LegacyNet participation. Here we list the six highest priority multi-site papers.

#	Research question	Required measurements
1	Does the inclusion of plant diversity in multispecies mixtures increase yields and/or lead to increased yield stability across environments? (yield comparison with high-nitrogen plots)	• Biomass yield in grassland ley • Botanical composition in grassland ley • Soil temperature and moisture • Soil type • Biomass yield in high-nitrogen G1 plots
2	Effect of sown grassland composition on biomass yield in the follow-on crop	• Biomass yield in follow-on crop
3	Nitrogen replacement potential of multispecies mixtures in crop rotation	• Biomass yield in grassland ley • Biomass yield in high-nitrogen G1 plots • N concentration in harvested biomass of plots in grassland ley • Species composition in grassland ley • Biomass yield in follow-on crop • N concentration in harvested biomass of follow on crop
4	Weed suppression potential of multispecies mixtures in grassland	• Species composition and mixture effects on weed biomass in grassland ley
5	Effects of multispecies mixtures on multiple ecosystem functions (multifunctionality) in a grassland ley and follow-on crop	• Biomass yield in grassland ley • Nitrogen yield of grassland ley • Forage quality • Weed biomass in grassland ley • Legacy effect on yield of follow-on crop • Legacy effect on nitrogen yield of follow-on crop • Species composition (persistence)
6	Species identities - Benefit of increased species richness or functional redundancy?	• Biomass yields in grassland ley • Biomass yields in follow on-crop • Soil temperature and moisture • Soil type