Midas

MARGINAL LANDS, INDUSTRIAL CROPS AND INNOVATIVE BIO-BASED VALUE CHAINS

Deliverable 3.6

Impact assessment on biodiversity at field scale (1st version)

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Executive summary

For the cultivation of the crops, MIDAS is investigating new innovative cropping systems (intercropping and agroforestry) that strengthen ecosystem services, notably biodiversity, in combination with biomass production for biobased applications (e.g. biochemicals, biomaterials).

In this report we present the field methodology manual developed for this project and the preliminary results from the first field season in 2023 aimed at evaluating the potential contribution to insect biodiversity of novel innovative cropping systems in agricultural landscapes.

We investigated insect biodiversity in strip-cropping systems on marginal lands in three field trials in Serbia, Spain, and Italy, with comparisons to adjacent monoculture fields used as reference sites. The field crops are cultivated for biomass production, using innovative cropping systems like intercropping while supporting biobased applications, such as biochemicals and biomaterials.

We measured number of flowers and number of flowering species, insects were collected using sweep nets, malaise traps and pitfall traps, with species identified through DNA metabarcoding methods.

Results from 2023 show that the species richness observed in sweep net catches was consistently higher in strip-cropping systems compared to monocultures. Although results from malaise and pitfall traps were mixed, the findings indicate that strip-cropping systems may foster a more diverse insect community, including pollinators and other beneficial species. A video of the collection of information during the first year field data collection was also made: MIDAS Assessing biodiversity in marginal land

A comparison of results between years will be included in the presentation at EUBCE in July 2025. These findings have significant implications for sustainable biomass production, policy development, and market prospects, particularly within the context of EU sustainability goals. The results also provide a further understanding of methodologies to measure impacts of different cropping systems, such as agroforestry and row cropping, on insect biodiversity.

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1 Introduction

1.1 Aim and context

This deliverable presents the approach, the field manual and the intermediate results of work done in MIDAS aimed at evaluating the potential contribution to insect biodiversity of the introduction of novel innovative cropping systems in agricultural landscapes.

The crops selected for MIDAS are grouped in oilseeds, lignocellulosic and dryland shrubs and include annual (castor, crambe, safflower, sorghum, hemp, carinata), perennial (miscanthus, cardoon, and lavender), fibre (hemp, miscanthus) and woody species (willow, poplar and Siberian elm). These crops are cultivated in innovative cropping systems that include both annual and perennial crops through practices like intercropping and agroforestry. The specific potential ecosystem services tested cover enhanced pollinator support and the cultivation of crops with high resistance or tolerance to pests and diseases, reducing the need for pesticides.

Two strip cropping systems have been developed for which field trials were established; an agroforestry system and an intercropping system. The agroforestry system is a combination of short rotation coppice and herbaceous crops and the intercropping system comprises of perennial and annual herbaceous crops. Biodiversity measurements are carried out at three intercropping case study locations, namely Soria in Spain, Bologna in Italy and Novi Sad in Serbia. To be able to measure the contribution to biodiversity of introduction of these novel crops in intercropping practices data on flying and ground dwelling insects are collected in the field trials, but also on nearby control fields. The latter relate to crops and cropping systems that are most typical in the local area.

In this report we present the field methodology manual developed for this project and the preliminary results from the first field season in 2023.

1.2 Reading guide

In chapter 2 the field work manual is presented. In chapter 3 the preliminary results are presented from the field insect observations in terms of flower counts, species richness and diversity identified through DNA metabarcoding method. The last chapter discusses next steps in the insect biodiversity measurements and expected final results.

2 Field work manual Midas

2.1 Introduction

In the following the manual is presented as used in the field work in in 2023 and 2024 (the future tense is used because this manual was developed in advance of the experiments). The 2023 field trials were the first and small adaptations were made to the manual based on the experience with the field trials in 2023. Beside the manual a video was also made of the field data collection in the site of UNIBO in the Bologna province MIDAS Assessing biodiversity in marginal land.

2.2 Cropping system per location

For task 3.2.2 of MIDAS, the insect biodiversity will be monitored at three trial locations of the project (Bologna in Italy, Novi Sad in Serbia and Soria in Spain) in the trial field and in a control field (see Figure 1 and Table 2). One field will comprise the innovative strip cropping system and the other field will comprise a typical crop rotation for the area. Measurements started in 2023 and will end in 2025.

Table 1 Cropping system per location per year

2023	Try-out year fieldwork by WENR + Develop methods and learning materials for transect counts, research into ecological interpretation of first results Workshop for local researchers to perform transect counts + effect monitoring
2024	Monitoring by local researchers
2025	Monitoring by local researchers
2026	Interpretation and reporting results

Table 2 Cropping system per location in 2023 and 2024

Country, participant	**Strip cropping**	**Typical crop rotation**	**Dates for collection or visit-ation**
2023			
Serbia, IFVC	Perennial (miscanthus) Annual (crambe, safflower and hemp or cas-tor in rotation)	Maize	1st collection: June 30- July 7, 2023 2nd collection: end July- Au-gust 3, 2023
Italy, UNIBO	Perennial (miscanthus) Annual (crambe, safflower and hemp or cas-tor in rotation)	Alfalfa	1st collection: June 25-30, 2023
Spain, CIE-MAT	Annual (crambe, safflower and melilotus of-ficinalis 1st year in rotation) Perennial Lavender	Cereal (wheat)	1st collection: July 17-21, 2023. 2nd collection: July 31-August 4
2024			
Serbia, IFVC	Miscanthus, Safflower, Crambe Sorghum, Castor	Barley?	Depends on weather
Italy, UNIBO	Perennial (miscanthus) Annual (crambe, safflower and hemp)	Alfalfa	collection: end of June
Spain, CIE-MAT	Rotation (crambe, safflower and melilotus officinalis (1st/2nd year in rotation) Perennial Lavender	Sunflower or fallow	Late July +(depends on fallow or sun-flowers)

Figure 1 Pictures of three field trial locations

1. Bologna

Strip crops: crambe, safflower and hemp

Control: alfalfa

2. Soria

Strip crops: crambe, safflower, melilotus, lavender

Control: winter wheat

3. Novi-Sad

Strip crops: white mustard; safflower; sorghum

Control: maize

2.3 Monitoring methods

Four different monitoring methods and data collection approaches were applied in every field trial location:

- Transect counts for pollinators
- Flower counts for flowers (the same transect as pollinators)
- Malaise traps for other flying insects
- Pitfall traps for crawling insects (mainly carabids and predatory spiders)

In 2023 in Spain and Italy all measurements will be performed during a one-week field visit by WENR accompanied by at least one local specialized field technician. Serbia will perform two monitoring weeks (once with, once without WENR). Chapter 3 will elaborate on each monitoring method. Figure 2 gives an insight in the preferred field design. Exact field design might be subject to improvisation since the design of the cropping system is not yet available for each location.

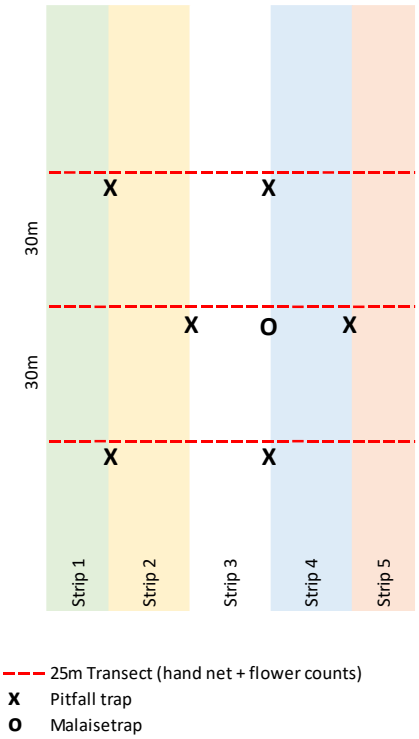


Figure 2 Schematic field design with preferred location of measurements (not to scale)

In the following Table 3 an overview is given of the material needed for every field trial in every of the 3 locations.

Table 3 Materials list (for collection in two field sites)

Item	Quantity	Used for	Trap use
Malaise SLAM Trap II by Bugdorm	4	catching flying insects, 2 at conventional cropping site, 2 in strip cropping site	malaise
50 ml centrifuge tubes with conical bottom screw cap DNase free with tube rack (e.g.: VWRI525-1072)	50	storage of collected insects	malaise, pitfall, hand net
2:1 Propylene glycol solution with water and 1% soap (to reduce surface tension)	4.2L	fluid for inside traps: 150 ml per malaise or pitfall trap	malaise, pitfall
95% ethanol for in field	1L	For storing transect catch in 50ml tubes	Hand net
20-30 cm metal pegs	64	4x4 for securing malaise trap corners and guy lines + 24*2 for pitfall trap lids	malaise, pitfall
Guy lines	16	4 per malaise trap for securing malaise corners	malaise
Hammer	1	To place metal pegs	malaise, pitfall
Waterproof marker	as needed	for labelling pots	all
Preprinted (NOT WITH INKJET PRINTER) sample labels (make sure if written by hand: in pencil otherwise will be removed with ethanol)	for each 50ml collection tube	for organizing catch	all
1L plastic containers with a diameter of 10cm with lids	24	pitfall traps	pitfall
raincovers for pitfalls 15x15cm (wood or plastic)	24	rain and vermin protection	pitfall
Soil auger (11cm width) (petrol driven when needed)	1	to drill holes in soil	pitfall
Spade	1	to help make holes in the soil	pitfall
Pickaxe	1	to help soften the soil	pitfall
Butterfly net	2	to catch flying insects	hand net
timer	1	to control time spent at each transect	hand net
field note book	1	take notes	Flower counting
killing jar	2	to kill flying insects	hand net
Tissue paper	as needed	for inside killing jars- to absorb ethyl acetate	hand net
entomological Leonard tweezers	2	for handling (dead) insects	pillfall, hand net
Paint sticks or soil markers/flags	12	For marking the start and end of sub-transects for walking	Hand net
Ethyl acetate (nail polish remover, acetone free!)	1 L	killing agent (for killing jars)	hand net
measuring tape 25m	1	to measure transect lengths	hand net
Ethanol >95%	5-10L	for washing (70%ethanol) and storing (95%) insect catches when back in lab	pitfall, malaise, hand net
- Medium Funnel	2-5	For separating insects from propylene glycol, and washing them with 70% ethanol for before storage in 95% ethanol	pitfall, malaise
a fine mesh fabric (<0.2mm²)	Wide enough to cover the medium funnel	For washing the insects of propylene glycol and transferring them to storage tubes	pitfall, malaise
Storage containers (10L)	2	To collect used propylene glycol, mix from traps and ethanol from washing	pitfall, malaise
Wash bottles	2-5	For washing insects with 70% ethanol and for filling sample tubes	Pitfall, malaise
Small funnel	2	To fill DNASE-free plastic storage tubes with 95% Ethanol	pitfall, malaise, hand net

2.4 Sampling methods

Preparation for trap setting:
Before entering the field mix a solution of 70% propylene glycol solution with a 1% soap. Bring at least 4.2L of this solution to the field or prefill each pitfall trap with 150ml of this solution before entering field. Use this solution for the pitfall traps and malaise traps (150 ml per trap).

Sample labelling
It is very important to use the following unique sample code for each sample:

- Country Abbreviation (Se for Serbia, It for Italy, Sp for Spain) +
- year +
- type of trap (PIT, NET, or MAL) +
- cropping system (S for strip cropping, T for Traditional cropping) +
- replicate (1 or 2) and trap number (1-3 for sweep nets, 1-2 for malaise traps and 1-6 for pit-fall traps)

For example:
Se23_PIT_S1.6 = Serbia, 2023, Pitfall trap, Strip cropping, Replicate 1, trap 6
It24_MAL_T2.2 = Italy, 2024, Malaise trap, Traditional cropping, Replicate 2, trap 2
Sp25_NET_S2.3 = Spain, 2025, Net trapping, Strip cropping, Replicate 2, trap 3

ITALY: Bologna (or more accurate place name)
44°29'22.79" N 11°20'20.40" E
Malaise: It24_MAL_T2.2
Collection date: 25 June, 2023
Collector: John Doe, UNIBO

This is an example of a label- the most important part is **the CODE in bold**.

Each year draw a map of the locations of each trap in the field.

Malaise trap method

1. Assemble the malaise traps (except for the bottle with propylene glycol) at the side of the field. It is easier to first assemble the traps before transporting them to the right location in the field. Also fill the trap bottle with propylene solution, but do not attach to trap yet (carry to field location with lid on).
2. Transport each malaise trap and other materials (guy lines, metal pegs, hammer, trap bottle) and to the trap location (figure 1- maise traps marked with “o”).
3. Fix the traps at all four corners of the tent with the metal pegs. It is crucial to firmly secure the malaise trap, as the traps are placed in open fields where they catch a lot of wind and would otherwise easily be blown away.
4. Attach 4 guy lines from the malaise trap to the soil to further strengthen them and fasten to the ground with metal pegs.
5. Screw the bottle filled with propylene glycol:water (2:1) to the bottle already attached to the malaise trap *(approx. 100 ml)*. Make sure the bottle is correctly positioned (see figure 2)! Tie the top of the tent to the bottles and close the zipper around the bottle. Make sure not to spill the fluid.
6. Leave the malaise traps in the field *for 5 days*.
7. If there is a storm coming up (> 5 Bft), do not place the malaise traps (or remove them prior to the storm and replace them afterwards). The malaise traps are not made to withstand strong winds and they might break.

8. Take a picture of each malaise trap plus its direct surroundings. That will come in handy later for explaining results.
9. Collecting the malaise trap: *put a (pre printed) paper label in the bottle*. Keep the bottles filled with fluid *upright,* as they are not fully watertight.
10. Process and store samples according to chapter 4.

Figure 3 *Malaise trap, with a close-up of the collection bottle on the right picture.*

Pitfall traps

1. Use the soil auger to make holes in the soil large enough to fit the pitfall traps - about 11cm diameter, 15cm deep
2. Place the traps according to the schematic field design figure 1 (or according to the plan for your specific field, each pitfall trap is given a replicate number), with two rows of 3 pitfall traps on either side of the malaise trap. Make sure the edge of the pitfall trap is flush with the soil surface and not sticking out above the surface- otherwise insects will not fall in.
3. Cover traps with the square cover about 2 cm off the ground by placing 2 metal pegs at an angle into the soil to protect the trap from rain, leaving room for ground insects to enter, but not small vertebrates.
4. After 5 days in the field, place a pre-printed sample label (with the unique trap number and date) into each pitfall trap and cover the trap with the lid.
5. Remove the pitfall trap from the soil.
6. Take the traps back to the lab for further handling. Keep the traps upright when transport-ing!

NB: Make sure to process samples in the lab for storage in >95% ethanol (See Chapter 4) within 1 day after bringing the bottles from the field. If this is not possible, store the traps temporarily in a fridge (4°C)

Transect pollinator counts

To cover the whole cropping system with the transect pollinator counts, the transects will be split up in three sub-transects that lie perpendicular to the direction of the cropping strips (see figure 1). Each transect will be 25 meters long. Each transect spans 2 meter wide. The sub-transects lie at least 20 meters apart, if possible. Include the exact location of the transect in the map of the spe-cific field (map should also include locations of malaise and pitfall traps)

Weather circumstances have to be sunny (maximum of 25% cloud cover), temperature minimum of 18˚C, maximum 35 ˚C, wind maximum 4 Beaufort (raises dust and loose paper; small branches moved), no precipitation.

Preparation for transect walking:

1. At least 1 week before sampling, clear a 1 m wide path through the strip-cropping field for the each sub-transect. If necessary, in the traditional field as well (for example for maize where transect walking could be difficult)
2. Measure the start and end points of the sub-transect, making sure the center of the transect is in the centermost cropping strip.
3. Prepare the preservation pots and the killing pot: Cover the bottom of the pot with tissue paper. Add a tablespoon of Ethyl acetate and close the lid. Use nail polish remover without acetone, as acetone will cause the pollinator's bodies to become brittle.

Walking the sub-transect (3 sub-transects per malaise trap):

1. Start the timer for 5 minutes.
2. Walk up and down the length of the sub transect, sweeping the field while capturing as many pollinators as possible and holding them trapped within the net, until the timer runs out. If interrupted, stop the timer and/or add any missed amount of time at the end.
3. Once the time is up, sweep vigorously to collect catch at the tip of the net.
4. Place the lower tip of the net, containing all captured pollinators, in the killing pot and close the lid as much as possible around the catch. Wait until the catch is suitably sedated to be extracted (use the time to walk another transect). If there is still visible movement within the pot after several minutes, consider adding more Ethyl acetate. Exposure of the pot to sunlight can also expedite the process. See this video for another idea of how to transfer insect to the kill jar
4. use the soft insect tweezers to transfer the insects to the 50ml centrifuge tube.
5. Add sample label (including the transect code) and 95% ethanol to the centrifuge tube.
6. Preservation of the samples: Refrigerate the preservation pots as soon as possible at -3 to -7°C.

Flower count transects

After the first pollinator survey all flowers should be counted (or estimated when large numbers) by species in each whole sub-transect of 25m. Count all flowers on each side of the sub-transect line (within 1m wide from each side of the transect).

Only flowering specimens, except for grasses, will be included in the count. For each species the unit of count should be noted so the flower structure e.g. composite flower (sunflower), ear (cereal), umbel (carrot), head (single flower or composite like sunflower or for example lathyrus).

Transect identification code:		Date:	Details: e.g. weather	Surveyor:
Species name	**Unit of count**	**Average size per unit**	**Number of flowerunits**	**Details**
Achillea millefolium	*Umbel*	*30 mm Ø*	*40*	*…*
Trifolium repens	*Flower head*	*10 x 10 x 10 mm*	*100*	*…*
Lathyrus pratensis	*Cluster*	*40 x 20 x 20 mm*	*20*	*…*
Helianthus anuus	*Flower head*	*120 mm Ø*	*10*	*…*
…	…	…	…	…

Walk up and down the length of each subtransect and note the number of flower units and unit of count (see table above for example) for each flowing species in the subtransect. Also note the average size of each unit of count, using diameter for disc-shaped, flat-topped units such as umbels and solitary flower heads, and width x length x height for other units, see examples above). Record only average sizes from 5 measured flowers for each flower species.

Washing and storing samples from pitfall and malaise traps

Method:

1. Place the sieve mesh fabric (size ~0,2mm) over the medium funnel, and the funnel inside the sink or 5-10L jerrycan
2. Pour the content **AND LABEL** of the pitfall or malaise trap into the sieve mesh covered funnel.
3. Remove all larger debris or non-insect specimens from the mesh (e.g., leaves, slugs or earthworms)
4. Wash the insect catch with 70% ethanol while gathering the catch in the center of the mesh, making sure all insects are washed move and pick them up with the soft insect tweezers.
5. Transfer the insect catch **AND LABEL** from the sieve mesh into the 50ml storage tube** with soft insect tweezers.
6. Fill the 50ml storage tube with **95% ethanol** to cover the insects and label contents. Do not fill the tube to the top if not necessary. For sending, do not add more than 30mL of ethanol.
7. Make sure the sample label from the trap was correctly transferred to the storage tube- and screw on the cap of the storage tube.
8. Store the tube in fridge or freezer until further processing (DNA analysis)
   -if further processing takes place after <1 week → store tubes in fridge (4°C)
9. Wash sieve, funnel and tweezers with a bit of ethanol and then more thoroughly with either demineralized water (preferred) or tap water to remove any remaining insect traces, prior to use on a next sample
10. Continue with step 1-9 for each sample (pitfall or malaise)

**if the number of insects is too large for one 50ml tube, divide the content over two or multiple 50ml tubes (use same sample code, but name tubes with e.g. subcode A, B, C). *Storage of samples in another type of tube or bottle, is possible, for instance if the number of insects per sample or the size of some of the insect specimens themselves, is too large for one 50ml tube.*

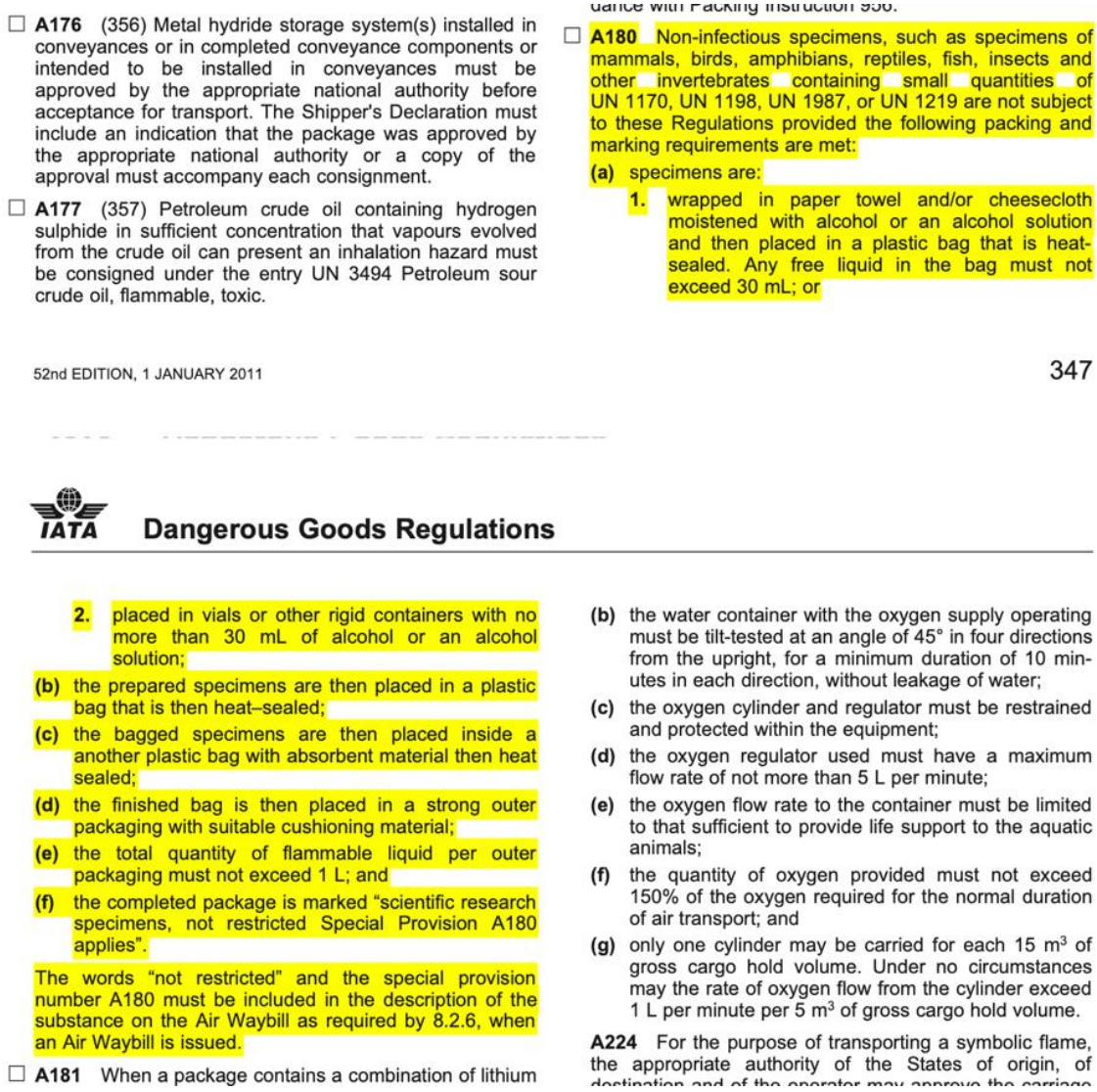
Limit the amount of ethanol in the bottle to 30ml: the insects should be just completely covered, but don't fill the bottle to the top.

Shipping samples to the Netherlands

If you are sending samples to Wageningen from other countries, we will provide the shipping labels.

1. Email us the following information to postkamer.fb@wur.nl with CC jenny.lazebnik@wur.nl or gilian.vanduijvendijk@wur.nl
   a. a note including "We would like to send samples to Wageningen Environmental Research at cost of the MIDAS project (Project number: 101082070)"
   b. Sender address including your contact name, institute, department, and postal details
   c. Number of boxes, weight of the boxes, size of boxes

2. We will then send <u>you two documents</u> and instructions for scheduling pickup. One is a word document (special provision A1) to stick to the outside of the box) and another is a pdf containing the shipping label (see examples in link)
3. Pack up the samples carefully (See Figure 4 below for HOW according to the A180 provision) and label the shipping box with the upright orientation. (**note: no be more than 30 ml ethanol per single sample tube)**



☐ **A176** (356) Metal hydride storage system(s) installed in conveyances or in completed conveyance components or intended to be installed in conveyances must be approved by the appropriate national authority before acceptance for transport. The Shipper's Declaration must include an indication that the package was approved by the appropriate national authority or a copy of the approval must accompany each consignment.

☐ **A177** (357) Petroleum crude oil containing hydrogen sulphide in sufficient concentration that vapours evolved from the crude oil can present an inhalation hazard must be consigned under the entry UN 3494 Petroleum sour crude oil, flammable, toxic.

dance with Packing Instruction 956.

☐ **A180** Non-infectious specimens, such as specimens of mammals, birds, amphibians, reptiles, fish, insects and other invertebrates containing small quantities of UN 1170, UN 1198, UN 1987, or UN 1219 are not subject to these Regulations provided the following packing and marking requirements are met:

(a) specimens are:

1. wrapped in paper towel and/or cheesecloth moistened with alcohol or an alcohol solution and then placed in a plastic bag that is heat-sealed. Any free liquid in the bag must not exceed 30 mL; or

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IATA Dangerous Goods Regulations

2. placed in vials or other rigid containers with no more than 30 mL of alcohol or an alcohol solution;

(b) the prepared specimens are then placed in a plastic bag that is then heat–sealed;

(c) the bagged specimens are then placed inside a another plastic bag with absorbent material then heat sealed;

(d) the finished bag is then placed in a strong outer packaging with suitable cushioning material;

(e) the total quantity of flammable liquid per outer packaging must not exceed 1 L; and

(f) the completed package is marked "scientific research specimens, not restricted Special Provision A180 applies".

The words "not restricted" and the special provision number A180 must be included in the description of the substance on the Air Waybill as required by 8.2.6, when an Air Waybill is issued.

☐ **A181** When a package contains a combination of lithium

(b) the water container with the oxygen supply operating must be tilt-tested at an angle of 45° in four directions from the upright, for a minimum duration of 10 minutes in each direction, without leakage of water;

(c) the oxygen cylinder and regulator must be restrained and protected within the equipment;

(d) the oxygen regulator used must have a maximum flow rate of not more than 5 L per minute;

(e) the oxygen flow rate to the container must be limited to that sufficient to provide life support to the aquatic animals;

(f) the quantity of oxygen provided must not exceed 150% of the oxygen required for the normal duration of air transport; and

(g) only one cylinder may be carried for each 15 m^3 of gross cargo hold volume. Under no circumstances may the rate of oxygen flow from the cylinder exceed 1 L per minute per 5 m^3 of gross cargo hold volume.

A224 For the purpose of transporting a symbolic flame, the appropriate authority of the States of origin, of destination and of the operator may approve the carriage

Figure 4 Instructions for the shipping of the catch to the Netherlands

3 Results of 2023 field work

3.1 Flower counts

Flower counts indicated greater species richness in strip-cropping fields than in adjacent monoculture fields, supporting potential for a higher diversity of flower-dependent insects in strip-cropping. Indeed, sweep net sampling consistently indicated greater species richness in strip-cropping systems across all countries (Figure 5).

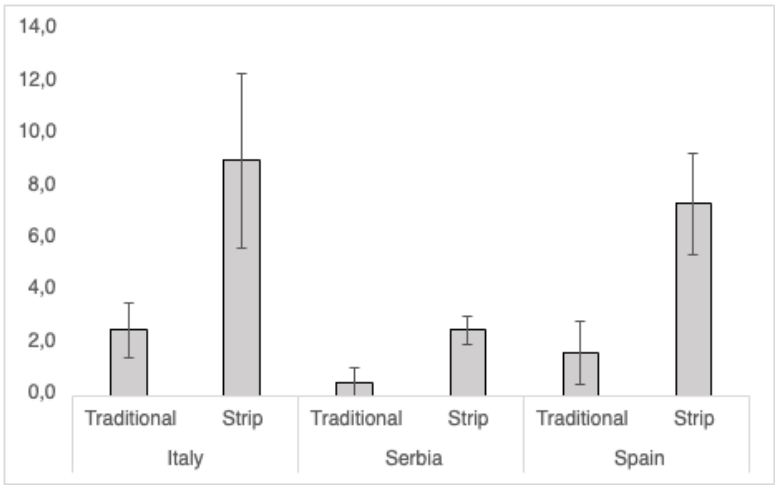

Figure 5 Mean flower species per country 2023, N=6 for each bar

3.2 Biomass

As shown in Figure 6, biomass differed greatly between countries. Highest values were found in Serbia for both malaise traps and pitfall traps, yet no clear differences were found between field types. In Spain, the strip cropping field showed a significantly higher biomass than the conventional monocultural field, for both trap types. In Italy, biomass only differed between field types for pitfalls, with much higher values in the monocropping fields.

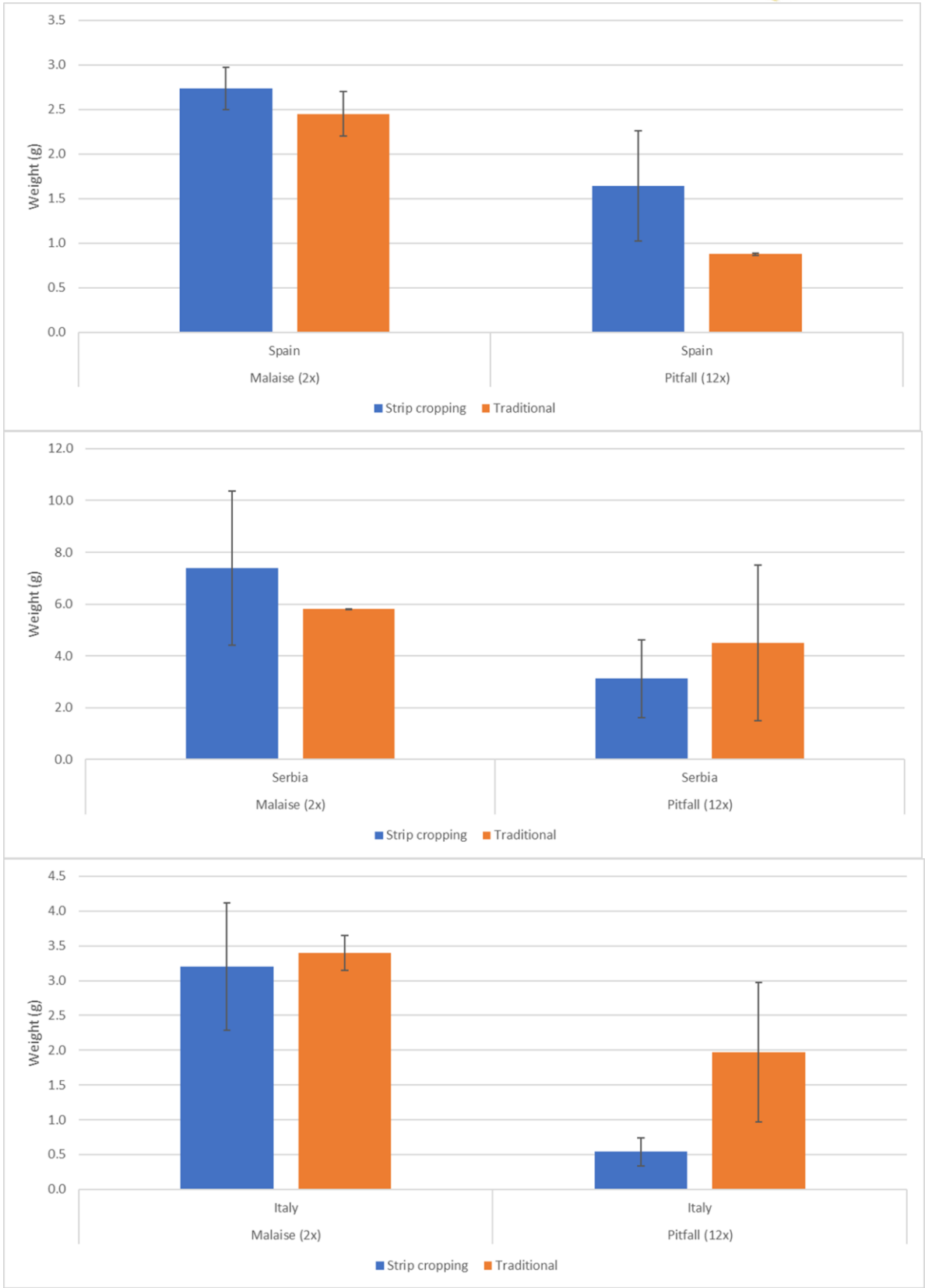

Figure 6 Average wet biomass per sample for samples from malaise traps (left) and pitfall traps (right), as collected in Soria (Spain, upper panel), Novi-Sad (Serbia, middle panel) and Bologna (Italy, lower panel)

3.3 Species richness

When biomass results are compared with results for taxonomic diversity as measured via DNA analysis, it becomes obvious that both measures do not fully correlate. Taxonomic diversity varied by crop type and location (Figure 7)

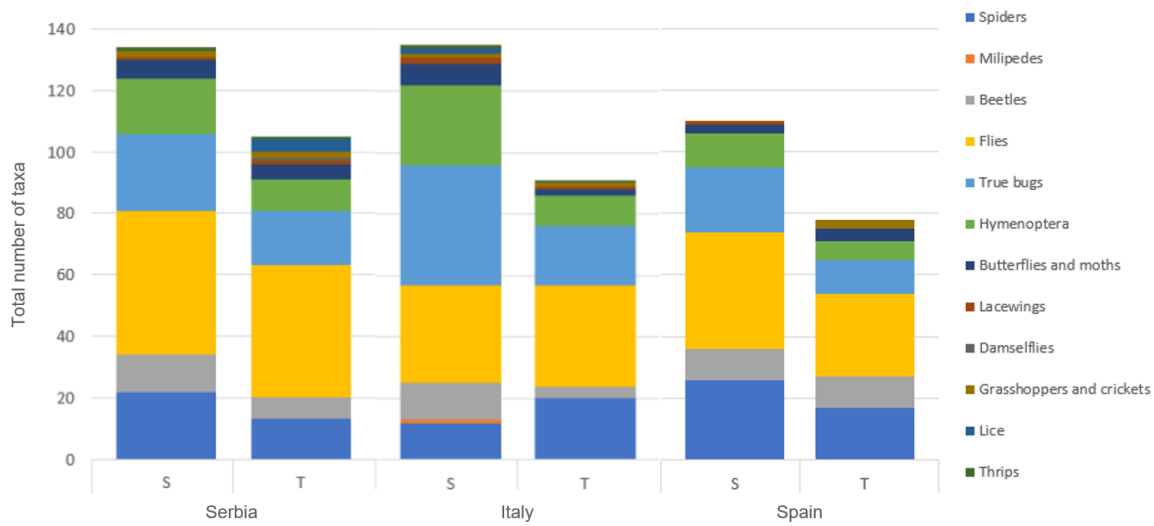

Figure 7 Total arthropod taxonomic richness (number of species in 12 transects) in the strip-cropping field (S) and the monocultural control field (T), per country

If we study diversity patterns in more detail per trap type (Figure 8), we see that biomass and species richness were correlated for pitfalls, but not for malaise traps (i.e. flying insects were abundant yet showed low diversity in some plots).

Interestingly, diversity observed via sweepnets was higher for strip cropping fields in all three countries.
Taxonomic orders, dominating in terms of the number of species they represent, were roughly similar among fields and countries (see Figure 8). However, these taxonomic order were clearly different between trap types, showcasing that different traps indeed inventoried different subsets of the local arthropod communities.

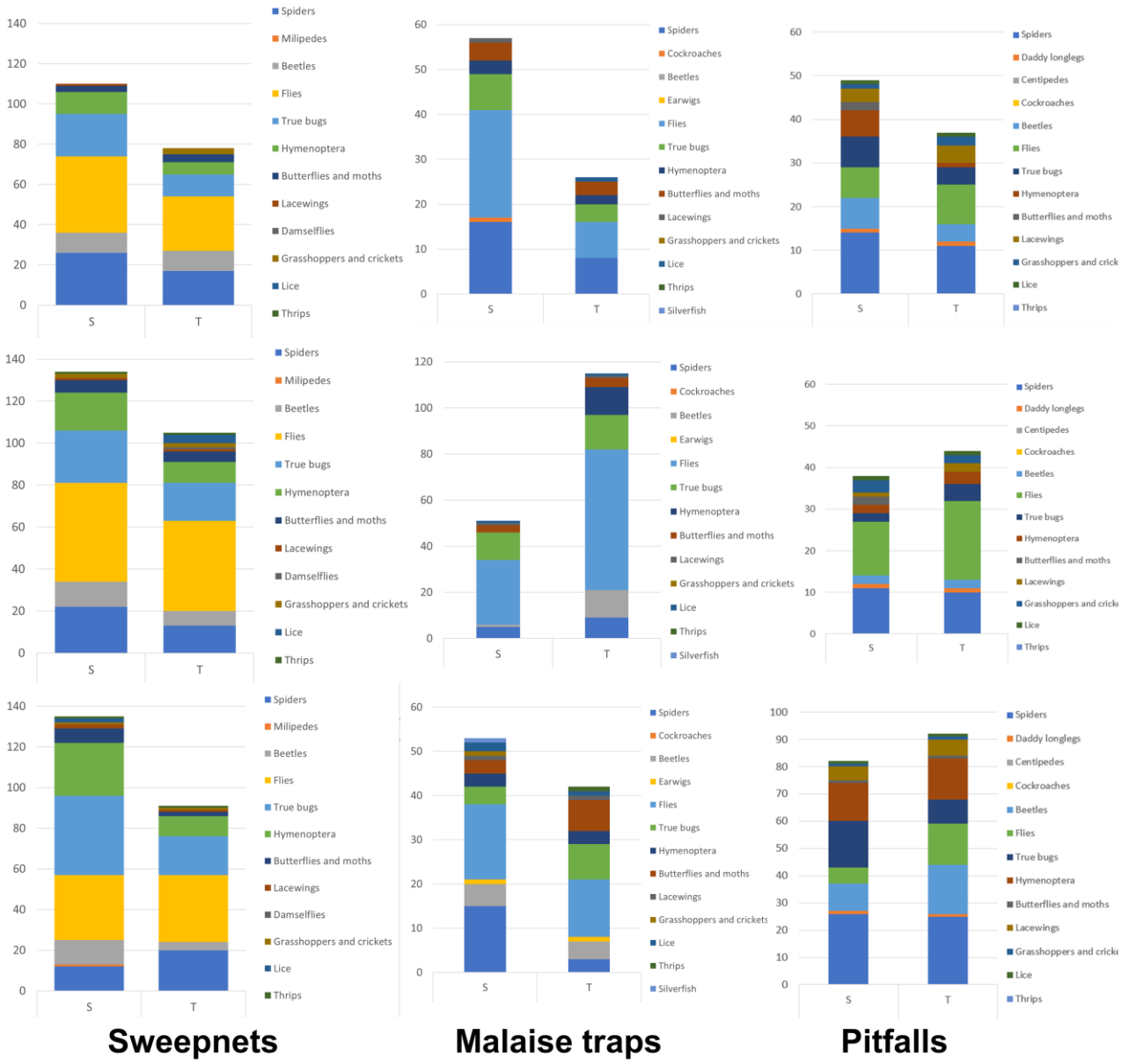

Figure 8 Taxonomic diversity, measured as the number of identified taxa per order in sweepnets, malaise falls and pitfalls for Soria (Spain, upper panel), Novi-Sad (Serbia, middle panel) and Bologna (Italy, lower panel).

3.4 Species composition

As shown in the NDMS plots in Figure 9, 10 and 11, every country has a very distinct insect community. Dots in the plot represent individual samples and samples per country (and per field for sweep net samples) are marked with a polygon. For sweep net samples (Figure 9, left panel), the polygons for field type partially overlap, while no overlap is present among countries. For malaise traps (Figure 10, left panel) and pitfall traps (Figure 11, left panel) a bit more difference between field types seems present, which can however well be caused by the low sample number per field type (N=2). None of the shapes formed by the community assemblages overlap when compared between countries.

The right panels in Figure 9, 10 and 11 depict the relative abundances of different arthropod orders, as obtained via identification of the arthropod samples by means of DNA metabarcoding. Thus, colours in the bars represent the percentage of DNA reads obtained from a sample that was assigned to taxa from a given order. Therewith, these figures provide further insight in the dominant orders and differences therein that caused the variation in community composition shown in the NDMS plots.

Communities obtained via sweepnets (Figure 9) were dominated by (DNA from) true bugs (Hemiptera) and flies (Diptera) in all countries. In Spain, beetles (Coleoptera) were more abundant than in the other countries, particularly in the monocropping fields. This field also harboured relatively many grasshoppers.

Communities obtained via malaise traps (Figure 10) also were dominated by true bugs and flies in most countries, except for the strip cropping field in Italy, where relative abundances of true bugs were much lower.

For pitfall traps (Figure 11) the community differences among countries were most markedly caused by shifts in dominant orders, with flies dominating in Serbia, grasshoppers and crickets in Spain and spiders in Italy.

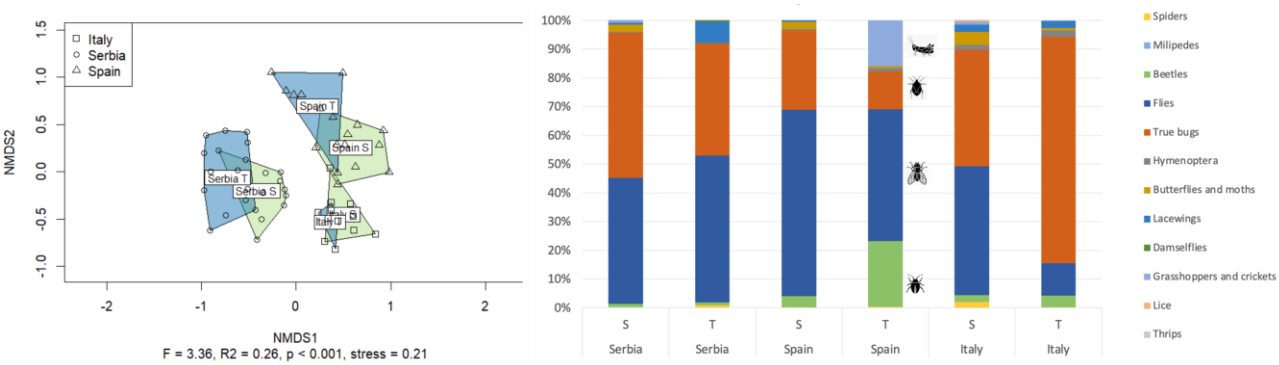

Figure 9 Results for species composition in sweep net catch per country per field (S = strip cropping, T = conventional monocropping). Left figure: NDMS plot, right figure: relative abundances (in terms of number of DNA reads) per order

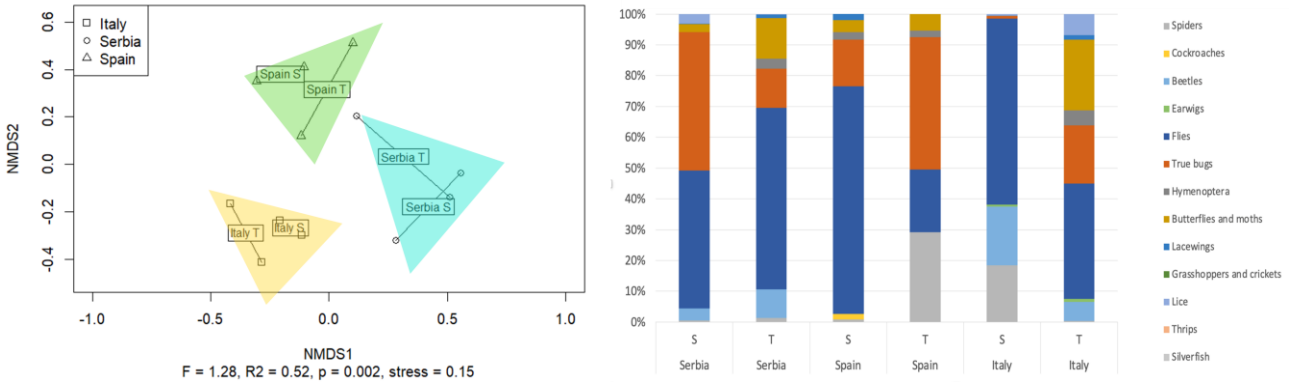

Figure 10 Results for species composition in malaise trap catch per country per field (S = strip cropping, T = conventional monocropping). Left figure: NDMS plot, right figure: relative abundances (in terms of number of DNA reads) per order

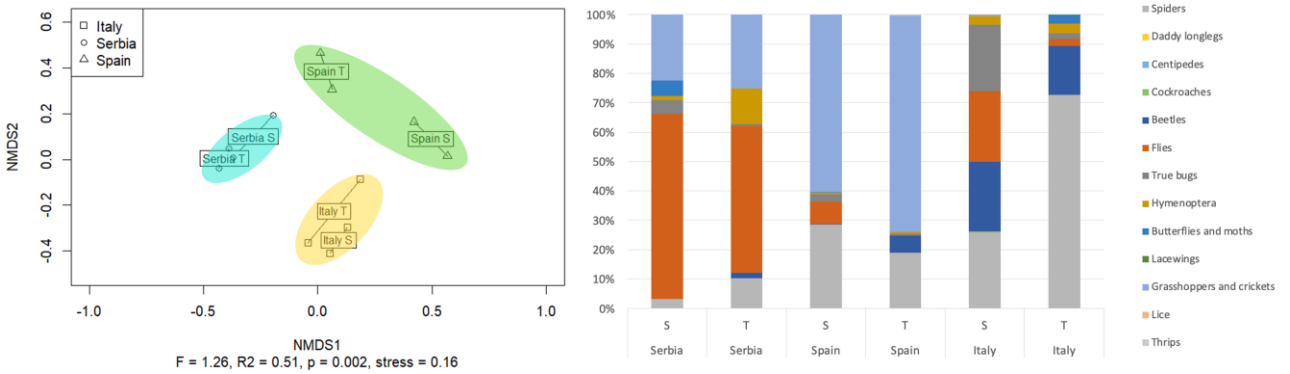

Figure 10 Results for species composition in pitfall trap catch per country per field (S = strip cropping, T = conventional monocropping). Left figure: NDMS plot, right figure: relative abundances (in terms of number of DNA reads) per order

4 Conclusions

Methodological insights

The first set of field trials led to some methodological insights. The timing of collection at each of the experimental fields was not always ideal. In the first season there were mismatches in maturing times of the crops used in the conventional field compared to the crops in the strip-cropping fields. For example, in Soria, while the safflower was just beginning to flower, the conventional wheat (control) field was ready to be harvested. Because of this, we agreed to change the field protocol, so that collections could be done at two different timepoints. At least one time point according to the blooming time of the strip crop (in 2024 the week that safflower was mostly in bloom). This was in order to standardize at least one important factor which may influence insect biodiversity. By standardizing the collection time with the timing of a common flower in the strip-cropping sites at all countries we are hoping to compare the general status of insect biodiversity at the cropping systems. Since the conventional field sites differ significantly between countries we can only address biodiversity at those sites when we can assume the biodiversity would be at its peak (so during a flowing or mature, but not completely dry) stage.

In the upcoming field trials, we are trying to take these insights into account within the limits of our budget. Ideally, we would have several sampling times in order to highlight the major benefit of using the strip-cropping methods for biodiversity- that there would be a much greater window op flowing plants in the field due to the crop-diversity and the range in growth-seasons of the diverse strip-crops. Unfortunately, due to the labour-intensive nature of this kind of sampling and subsequent a costly DNA analysis, we modified the protocol as mentioned above without adding more sampling periods.

Biodiversity insights

The distinct conditions of marginality at the different sites have likely played a role in the biodiversity community. For example, the high droughts (Spain) or heavy storms and flooding (Serbia) played a great effect on the dominant species in each country as well as the type of crops used as controls.

The findings highlight the biodiversity benefits of strip-cropping for sustainable biomass practices, should be aligned further with EU environmental resilience policies. In MIDAS the methodology to measure effects of strip cropping practices on insect biodiversity. The first results point in the direction of increased biodiversity in strip-cropping fields. This needs to be further analysed though with the next measurements in the MIDAS field trials in 2024 and 2025. The positive contribution of strip cropping points to market potential for sustainable biomass solutions adaptable across European agricultural landscapes offering a sustainable, biodiversity-supportive practice for biomass production.

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