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„Direct Insect Mortality by Different Mowing Practices: A Biomass-Based Quantification in Agricultural Grasslands"

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Abstract

Rapid intensification and change of agricultural used land are frequently identified as important causes for today's global biodiversity decline, including insects. Agriculture practices need to be as biodiversity friendly as possible if we want to preserve insect biodiversity and associated ecosystem functions. This study is therefore conducted to classify various common mowing techniques regarding their direct impact on insects in agriculturally differently managed meadows. To classify the different mowing techniques in terms of their negative effects on insects, the biomass of dead insects is tested as a potentially standardizable parameter. Insect biomass and orthopteran diversity of freshly mown plots of an intensively and an extensively managed meadow are compared. A main aspect is the evaluation of error rates regarding overseen individuals during the sampling, to determine the representativeness of the tested classification methods.

Results show that the choice of mowing technique used, has strong impact on the direct insect mortality and accumulated dead insect biomass in tested meadows. Proportion of dead insect biomass to total insect biomass per plot is significantly higher (Kruskal-Wallis H, $p < 0.001$, $n = 120$) if a rotary disc mower with conditioner (median = 21,25 %) is used in contrast to a double-knife cutter bar mower (median = 0,58 %) or a simple rotary disc mower (median = 1,96 %). Different time of day of mowing seems to have minor influence on the direct insect mortality as no significant differences were measured. Insect biomass does not differ significantly between the two studied sites. Diversity of Orthoptera in extensively versus intensively managed meadow plots was higher (in total 10 vs. 4 species) as was the number of individuals (104 vs. 38). The biomass of insects that were initially overlooked and subsequently discovered during laboratory re-examination of "emptied in the field" collection bags was compared to the total biomass of insects collected per plot, sampling time, and collection method. The resulting percentage, representing the error rate of overseen insect biomass, was consistently very low, with < 0.001 % in 95 out of 173 counts and less than or equal to 10 % in 145 of 173 counts.

The tested evaluation methods show sufficient robustness to allow objective comparisons and classifications, confirmed by the low error rate observed. Finally, biomass might serve as a valid and easily implementable classification parameter to evaluate the direct negative impact of different mowing techniques on insects.

Zusammenfassung

Die rasche Intensivierung und Veränderung der landwirtschaftlichen Nutzflächen, wird häufig als eine der wichtigsten Ursachen für den derzeitigen weltweiten Rückgang der biologischen Vielfalt, einschließlich der Insekten, genannt. Landwirtschaftlichen Praktiken müssen so biodiversitätsfreundlich wie möglich sein, wenn wir die biologische Vielfalt der Insekten und die damit verbundenen Ökosystemfunktionen erhalten wollen. Diese Studie wird daher durchgeführt, um verschiedene gängige Mähtechniken hinsichtlich ihrer direkten negativen Auswirkungen auf Insekten in landwirtschaftlich unterschiedlich intensiv bewirtschafteten Wiesen zu klassifizieren. Für die Einstufung der Beeinträchtigung der Insekten durch die unterschiedlichen Mähtechniken, wird die Biomasse toter Insekten als potenziell standardisierbarer Parameter herangezogen. Es werden die Insektenbiomasse und die Diversität von Orthopteren in frisch gemähten Parzellen einer intensiv und einer extensiv bewirtschafteten Wiese verglichen. Ein Hauptaspekte ist die Evaluierung von Fehlerquoten hinsichtlich übersehener Individuen während der Probenahme, um die Repräsentativität der getesteten Klassifizierungsmethoden zu bestimmen.

Die Ergebnisse zeigen, dass die Wahl der Mähtechnik einen starken Einfluss auf den Verlust an Insektenbiomasse in den untersuchten Wiesen hat. Der Anteil der toten Insektenbiomasse an der gesamten Insektenbiomasse pro Parzelle ist signifikant höher (Kruskal-Wallis H, $p < 0.001$, $n = 120$), wenn ein Scheibenmähwerk mit Aufbereiter (Median = 21,25 %) im Vergleich zu einem Doppelmesser-Balkenmäher (Median = 0,58 %) oder einem einfachen Scheibenmähwerk (Median = 1,96 %) verwendet wird. Ein unterschiedlicher Tageszeitpunkt der Mahd scheint einen geringen Einfluss auf den Verlust an Insektenbiomasse zu haben, da keine signifikanten Unterschiede gemessen werden konnten. Die Insektenbiomasse unterscheidet sich nicht signifikant zwischen den beiden Untersuchungsstandorten. Die Diversität der Orthoptere in den extensiv bewirtschafteten Wiesenparzellen war höher als in den intensiv bewirtschafteten (insgesamt 10 vs. 4 Arten), ebenso die Anzahl der Individuen (104 vs. 38).

Die Biomassen von Insekten, welche im Feld übersehen wurden und erst während der laboratorischen Nachuntersuchung von "im Feld entleerten" Sammelbeuteln entdeckt und gewogen wurden, wurden in Relation zu den korrespondierenden Gesamtbiomassen von Insekten pro Parzelle, Zeitpunkt der Probenahme und Sammelmethodik gesetzt. Die resultierenden Prozentwerte repräsentieren die Fehlerquote der Methodik und waren durchwegs sehr niedrig, mit < 0.001 % bei 95 von 173 Zählungen und kleiner-gleich 10 % bei 145 von 173 Zählungen.

Die getesteten Bewertungsmethoden werden, untermauert durch die geringe Fehlerquote, als ausreichend robust eingestuft, um objektive Vergleiche und Klassifizierungen zu ermöglichen. Die Insektenbiomasse könnte somit als gültiger und leicht implementierbarer Klassifizierungsparameter eingestuft werden, um den direkten negativen Einfluss verschiedener Mähtechniken auf Insekten zu erheben.

Keywords: agriculture, biomass, insects, mortality, mowing, orthoptera, quantification

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1 Introduction

Current data increasingly shows that rapidly intensifying agriculture, land use change and deforestation have the strongest negative impact on global arthropod diversity and abundance (Raven & Wagner, 2021). The scale of these threats can only be seized when one considers that humans and their domesticated animals nowadays account for 95% of the world's mammalian biomass (Bar-On et al., 2018). Accompanying that, approximately 40% of the terrestrial land area has been converted to cropland and pastures and still the human population is growing (Foley et al., 2005). While farmland expansion and intensification are the current solution to the increasing demand for food, they are one of the main causes of biodiversity loss and insect mortality (Newbold et al., 2015; Wagner, 2020). For this reason, there is necessity to make existing agricultural practices as biodiversity friendly as possible to mitigate their negative effects on insects and associated ecosystem functions (Seppelt et al., 2016; Poppenborg et al., 2019).

While the focus of agricultural key elements like mowing is on production effectiveness, their impact on biodiversity is often severe yet disregarded. For example, according to Fluri & Frick (2002) or Humbert et al. (2009) certain mowing methods differ in their negative effects on arthropods. In addition, longer intervals between mowing and/or later seasonal mowing offer some taxa, such as Hemiptera (Unterweger et al., 2017) or Hymenoptera (Buri et al., 2014) the possibilities to make (better) use of the increased available resources (Cizek et al., 2012). Likewise, flowers visited by Lepidoptera have the chance to bloom (Persson & Smith 2013) or oviposition and whole generation cycles can be completed (Walter et al., 2007). Thus, a more extensive mowing regime benefits certain insects and enhances local species diversity supported by past observations that biomass and biodiversity of intensively managed meadows generally is lower than of extensively managed meadows (Kruess & Tscharntke, 2002; Humbert, et al., 2009; Buri et al., 2014; Chisté et al 2016). Another aspect is that insect activity in general tends to increase with rising temperature. According to the time of day and weather, this leads to an increase (startling leads to higher damage through the mower) or decrease (startled insects can flee in time) of dead or severely damaged insects by mowing (Dover et al.,2010). However, many studies solely focused on a single taxon, such as bees or butterflies. Therefore, the possibility always persists that the focal taxon might be disproportionally affected compared to other occurring insect taxa (Buri et al., 2014). Moreover, Humbert et al (2009) highlight another problem arising in the context of such multifactorial and large-scale studies concerning the impact of meadow harvesting on fauna. Out of thirteen reviewed publications only half of the studies were of statistical significance. Together with insufficient replicability of the experimental design, available subject related information is still limited and a reproducible and reliable classification tool much needed.

Therefore, this master thesis is realized within the framework of a project commissioned by the Austrian Federal Ministry for Agriculture and Tourism, entitled “Erhebung von Grundlagen zur Bewertung insektenschonender Mähtechniken im Wirtschaftsgrünland und in artenreichen

Magerwiesen" [engl.: "Establishment of a survey method for the classification of mowing techniques regarding their impact on insects in agriculturally differently managed meadows"]. The Austrian Federal Ministry of Agriculture, Regions, and Tourism (BML) provided the funds through DaFNE (project nr. 101562). A major collaborator within above mentioned survey-method project is the Austrian Agency for Health and Food Safety (AGES) which also co-supervises this thesis.

One of the main aims of the above DaFNE project is to assess and quantify the effects of different mowing techniques to establish an evidence-based method for the quantification of insect damage through such mowing techniques. The project shall also investigate for potential differences among various meadow types i.e., intensively and extensively managed meadows. In Austria intensely managed meadows are characterized by strong fertilization input and high mowing intensity with several cuts per year (Tiefenbach et al., 2014), whereas extensively managed meadows only are harvested once or twice without much or any use of fertilizers (Buchgraber et al., 2011)

This master thesis investigates a biomass-based quantification of the direct insect mortality caused by five different mowing techniques which are tested simultaneously. Insects smaller than three millimetres are not included in this survey as their biomass is assumed to be a negligible proportion of total biomass and laboriousness of taxonomic identification would redundantly increase. Differences of insect biomass and Orthoptera diversity among one intensively (heavily fertilized, mown five times a year) and an extensively (unfertilized and mown once a year) managed meadow are compared.

The following hypotheses are addressed:

I. The five tested mowing techniques differ in the proportion of killed or severely damaged insects in relation to surviving insects. To test this hypothesis, I propose a potential evaluation method by weighing the biomasses of sampled dead or severely damaged insects and comparing them to the biomasses of sampled surviving insects after each mowing pass and for each sampled plot.
II. Biomass of insects in the grass clippings will be lower in the intensively managed meadow than in the extensively managed meadow. This hypothesis is based on other observations provided by literature, which indicate that intensive mowing practices contribute to habitat degradation, reduced resource availability and ultimately decreased insect biomass.
III. Based on existing literature suggesting a negative correlation between high mowing frequency and the abundance and diversity of occurring grasshoppers and katydids, the extensively managed meadow will have a higher number of species and greater abundance of Orthoptera compared to the intensively managed meadow.
IV. As insect activity generally increases with rising daytime temperatures, mowing at different times of the day (a.m. vs. p.m.) will impact the scale of the direct insect mortality caused by the mowing machinery. By comparing the biomasses of dead or severely damaged insects to the biomasses of sampled surviving insects, I propose that this is one way of evaluating this.

In addition, an important objective of this thesis is the evaluation of error rates concerning insect individuals - irrespective if dead or alive, though larger than 3 millimetres – that were initially missed during sorting out insects in the field and only found later in the laboratory while re-examination of the samples. The objective is to establish a reproducible and robust evaluation method for future objective comparisons and classifications of mowing techniques and their respective negative impact on occurring insects.

2 Materials and methods

2.1 Study area and period

Fieldwork was carried out between May and October 2022. The intensively managed meadow in Frankenmarkt has an annual mowing regime of four times thus sampling was carried out several times during the year: in spring (May 12, 2022), summer (July 13 and August 23, 2022) and autumn (October 5, 2022). The extensively managed meadow in Micheldorf is mown once a year and therefore was observed solely in summer (July 12, 2022).

The study was conducted on two meadows in Upper Austria (Figure 1), which differ greatly in their management intensity for herbage production. The meadows were selected beforehand by a committee of ecologists and farmers to be well representative of a typical intensively managed meadow and extensively managed meadow in Upper Austria. First site's location is Frankenmarkt (N 47.9937, E 13.411719) in the district of Vöcklabruck at an elevation of approximately 540 m above sea level. The meadow is intensively managed by the owning farmers, aiming to achieve a maximized production of forage for dairy cows and was mown four times in 2022. Thus, well representative of a widespread and dominant landscape type of Austrian agriculture (Buchgraber et al., 2011).

Location of the second meadow is Micheldorf in the district Kirchdorf (N 47.863380, E 14.132602) at an elevation of approximately 500 m above sea level. Due to several occurring plant species which are under full legal protection according to the Red List for Upper Austria (Hohla et al., 2009) i.e., *Lilium bulbiferum, Platanthera bifolia* or *Gentiana acaulis*, this former hay meadow has been integrated in a larger protected landscape since 2008 (Bejvl, 2010).

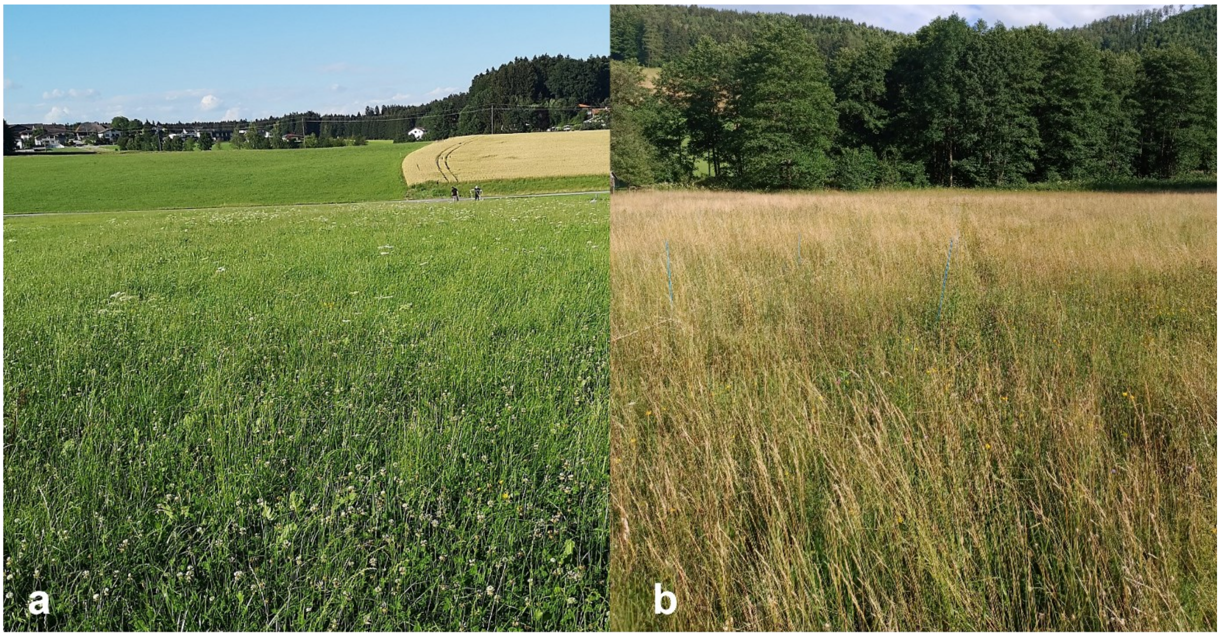

Figure 1. Meadow sites sampled in this study and photographed within two days in July 2022. (a) Intensively managed meadow in Frankenmarkt on July 13, 2022. (b) Extensively managed meadow in Micheldorf on July 12, 2022. (Photos: Dirk Louis P. Schorkopf)

2.2 Mowing and insect sampling techniques

The following five different mowing techniques were applied by staff members of the "Maschinenring Oberösterreich":

- Double-knive cutter bar system [ger.: "Doppelmessermähwerk"] (DCB)
- Rotary disc mower [ger.: "Herkömmliches Scheibenmähwerk"] (RD)

  Rotary disc mower with conditioner
  [ger.: "Scheibenmähwerk mit Aufbereiter"] (RDC)
- Rotary disc mower with conditioner and harrow
  [ger.: "Scheibenmähwerk mit Aufbereiter und Striegel"] (RDC1)
- Rotary disc mower with conditioner and deflective plate
  [ger.: "Scheibenmähwerk mit Aufbereiter und Abweiserblech"] (RDC2)

The designated meadow in Frankenmarkt was divided into two 53 x 45 m areas. One was mown in the late morning (09:00 - 12:00) and the other in the early afternoon (13:00 - 16:00) to investigate whether insect activity and mortality from mowing varied according to the time of day. The areas were mown in strips (53 x 3 m) for each of the five mowing techniques with spacing between strips. The position and distance of mown strips to each other was based on a pre-designed plan developed by AGES (see Appendix A). To avoid bias, the sequence of mowing techniques was alternated in new combinations every time an area was mown. However, in Micheldorf, the mowing pattern of the strips deviated from the original plan due to the shape of the meadow. Within each strip, three plots (3 x 3 m) were selected and sampled also according to the plan provided by AGES (see Appendix A) resulting in a total of 15 plots per area for the five techniques. Thus, a total of 30 plots (15 plots at a.m. and 15 plots at p.m.) were sampled each day the meadows were mown.

2.2.1 Insect and diversity

Immediately after mowing, cut vegetation was removed from the selected plots and sampled for insects using a sieving table including a collection bag installed underneath (3 l plastic bag). Following that, insects were sorted out according to their vitality status ("alive" or "dead or severely damaged") in the field and sorted into a corresponding vial (180 ml plastic sample container) for each plot. Alive individuals were being anaesthetised with a few drops of ethyl acetate ($CH_3CO_2CH_2CH_3$) to ease sorting out. Insects smaller than 3 mm are excluded from this survey as their biomass is assumed to be a negligible proportion of total biomass and extracting effort as well as determination of vitality would outweigh their potential benefit to this study.

To obtain a comprehensive record of all insects directly affected by the mowing, the ground surface of each plot was sampled in addition to cut vegetation. By using a cordless leaf vacuum cleaner (type: Echo ES-255ES; diameter: 135 mm) 20 randomly selected spots per plot were sampled for approximately two seconds each. The vacuuming targeted specimen that had fallen to the ground as a result of the mowing disturbance, dominantly ground-dwelling species and separated parts of damaged or killed insects. Collected individuals and insect parts were captured in a collection bag (3 l plastic bag) attached inside the vacuum cleaner. Determination and sorting in vials (180 ml plastic sample container) followed the methods as stated above.

The classification of "alive" or "dead or severely damaged" for cut vegetation as well as vacuumed ground surface resulted in four vials with collected insects per plot (30 plots/day), henceforth 120 samples per day of mowing. Figure 2 illustrates insects classified as dead or severely damaged. The total of five mowing days for the entire sampling period resulted in 600 samples. All samples were frozen immediately and transported in this state to the laboratory of the university of Vienna for preservation and further analysis.

Biomass of collected insects according to plot, sampling method (cut vegetation or vacuumed) and vitality status (alive or dead/severely damaged) was determined by weighing in an aluminium sample pan using a precision balance (Mettler Toledo AE200 Analytical Balance) with a readability of 0.1 mg. Diversity of insects was determined through classification of sampled individuals (by family/subfamily level, or by lower taxonomic rank if identification was unambiguous possible e.g. *Apis mellifera)*.

Figure 2. Various occurring insects which were highly impacted by the mowing machinery and therefore classified as severely damaged or dead. *Pseudochorthippus paralellus* female (a) and male (b) missing parts of their abdomens. (c) *Roeseliana roeselii* female decapitated and missing abdomen parts and legs. (d) *Apis mellifera* female worker missing the abdomen. (Photos: Johann Neumayer)

2.2.2 Orthoptera

Collected Orthopterans of each collection vial (four per plot) were weighed together with the above-mentioned precision balance. In addition, all individuals were counted for each plot and the body size (head to abdomen tip) of evidently undamaged individuals measured using graph paper with a grid of 1 mm and taxonomically determined to species rank.

2.3 Error rate concerning overseen insect individuals

To estimate the error rate i.e., insect individuals that may have been overseen during above-described evaluation technique (sorting of insects according to their vitality), collection bags were re-examined for any insect remains after sorting. To achieve this, bag content (grass material and soil) which had been examined for insects in the field and subsequently frozen, was weighed and placed in a large laboratory tray. The material was then carefully examined at least twice for any overseen insect fragments or whole individuals, using tweezers and a light source. In uncertain cases, such as seeds macroscopically resembling dead insects, a stereomicroscope (Wild Heerbrugg Typ 181300, 6,5x - 45x) was used for further examination. Thenceforth, the biomass of overseen insects/plot was placed in relation to the total biomass of collected insects per corresponding plot and time of sampling, resulting in a percentage representing the error rate.

2.4 Data analysis

Statistic analyzation as well as visualisation of the date collected was conducted with the statistic program R, version 4.2.2 (R Core Team, 2022). Boxplots, bar charts and histograms were generated to generated in R to visualize the distribution of the data. Descriptive statistics such as arithmetic mean, median, sum, min., max., and quartiles were calculated for individual datasets using Microsoft Excel (version 2019) and R (R Core Team, 2022). Additionally, depending on the research question, a Kruskal-Wallis H test or a Mann-Whitney U test was performed in R (R Core Team, 2022) to evaluate the statistical significance of plot-wise differences in insect biomass.

3 Results

3.1 Differences in dead insect biomass according to tested mowing techniques

The five tested mowing techniques (each: n = 24 plots) differed in the proportion of killed/severely damaged insects in relation to total insect biomass surveyed. With a lower median (0,6 % vs. ≈ 2 %) and slightly higher maximum value (24,56 % vs. 27,69 %) across the studied plots, the double-blade cutter bar mower, abbreviated as DCB, had a non-significantly lower impact compared to the simple rotary disc mower, abbreviated as RD. Percentage of impact was significantly higher by using the conditioner equipped techniques RDC, RDC1 and RDC2 with a median between ≈ 20 - 30 % for all three (Table 1 and Figure 3). The statistical analysis showed a significant difference among all five mowing techniques (Kruskal-Wallis H = 26,028; df = 4; $p < 0.001$; n= 120 - 24 plots per technique). For pairwise comparison of all tested mowing techniques, following the significant Kruskal-Wallis H test, a Dunn - Bonferroni test was performed. Pairwise comparisons showed (Table 2), that the biomass per parcel differs significantly between between DCB/RD and RDC, RDC1 as well as RDC2.

Table 1. Proportion of dead insect biomass (%) in relation to total insect biomass varied greatly depending on the mowing technique. The median, minimum, maximum, first quartile (Q1), and third quartile (Q3) values, expressed as percentages, are provided for each technique. The sample size for each technique was consistent, with 24 plots sampled per technique (n = 24). The statistical test, Kruskal-Wallis H, is included at the bottom to assess the overall significance of the observed differences. For a comprehensive understanding of "n" and mowing techniques, see figure caption of Figure 3.

Mowing technqiues and abbreviation (n = 24 each)		**median (%)**	**min (%)**	**max (%)**	**1Q (%)**	**3Q (%)**
double-blade bar mower	"DCB"	0,58	0,00	24,56	0,00	2,75
rotary disc mower	"RD"	1,98	0,00	27,69	0,44	9,33
rotary disc m. w. conditioner	"RDC"	21,25	0,00	96,27	4,83	47,75
rotary disc m. w. c. and harrow	"RDC1"	19,76	0,00	96,48	4,67	44,91
rotary disc m. w. c. and deflective plate	"RDC2"	29,86	0,00	95,27	11,68	49,12
Statistical test: Kruskal-Wallis H = 26,028; df = 4; p < 0.001; n = 120 (24 plots per technique)						

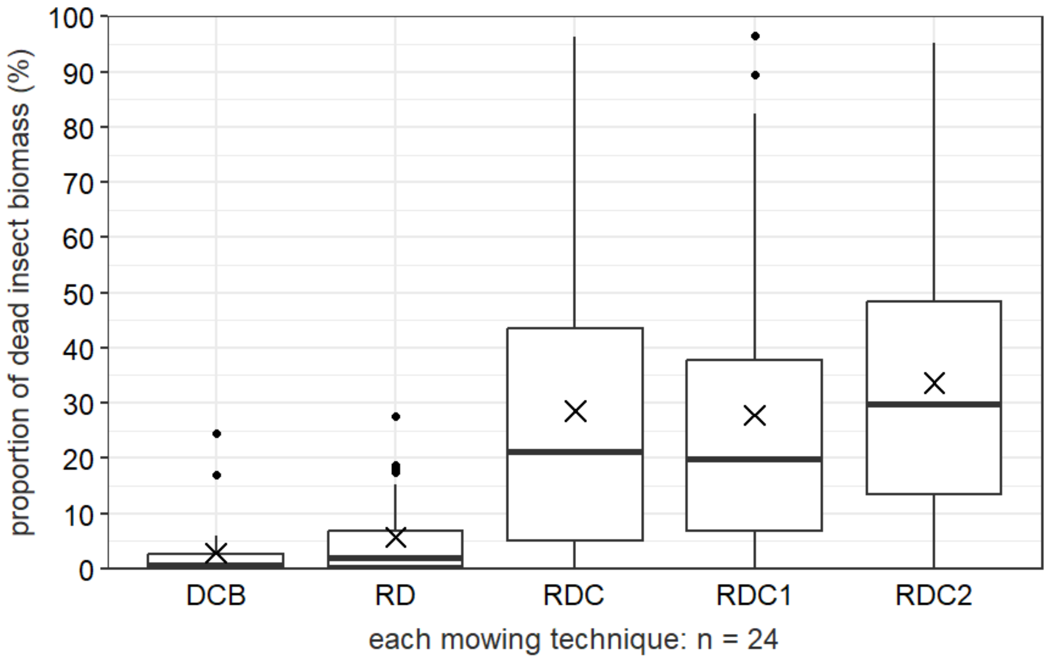

Figure 3. Significantly different mortality values caused by different mowing techniques. The y-axis represents the proportion of dead insect biomass relative to the total insect biomass, expressed as a percentage. The x-axis legend provides an explanation of each mowing technique: "DCB" for double-knive cutter bar system, "RD" for rotary disc mower, "RDC" for rotary disc mower with conditioner", RDC1" for rotary disc mower with conditioner and harrow "RDC2" for rotary disc mower with conditioner and deflective plate. "n" represents the sample size. In this case, the sampled plots for each mowing technique. Each day of mowing six plots per technique were sampled. Three in the morning and three in the early afternoon. Thus, a total of 24 plots for each of the five techniques were obtained across four sample days (6 plots x 4 sample days = 24 plots). The boxplots depict the distribution of dead insect biomass proportions for each mowing technique, with the median indicated by the horizontal line and the arithmetic mean indicated by the "x". Outliers, represented by individual points beyond the whiskers, are defined as values outside the 1.5 times the interquartile range. The results show significant differences in mortality values among the mowing techniques.

Table 2. Pairwise comparisons of all mowing techniques for the proportion of dead insect biomass to totacoloul insect biomass showcases significant differences between DCB/RD and RDC, RDC1 as well as RDC2. Pairwise comparison was conducted using a Dunn - Bonferroni test. The table presents the Z-score, unadjusted p-value (p unadj.), and adjusted p-value (p adj.) for each comparison. Significant differences between mowing techniques are indicated by a yellow background-colour with a significance level of $p > 0.05$.

Mowing tech. 1	-	Mowing tech. 2	Z	p unadj.	p adj.
DCB	-	RD	-1.135	0.257	1.000
DCB	-	RDC	-4.456	0.000	0.000
DCB	-	RDC1	-4.248	0.000	0.000
DCB	-	RDC2	-5.042	0.000	0.000
RD	-	RDC	-3.321	0.001	0.005
RD	-	RDC1	-3.113	0.002	0.009
RD	-	RDC2	-3.907	0.000	0.001
RDC	-	RDC1	0.208	0.835	0.835
RDC	-	RDC2	-0.586	0.558	1.000
RDC1	-	RDC2	-0.794	0.427	1.000
Statistical test: Dunn - Bonferroni					

3.2 Extensive vs. intensively managed meadow sites: Insect biomass differences

Surveyed total insect biomass (all detected insects of the cut vegetation and the vacuumed ground for each day and plot) in July was slightly, although not significantly (Mann-Whitney U test = 433; n = 30 plots; $p = 0.807$), lower in the extensively managed meadow than in the intensively managed meadow in Frankenmarkt on July 13, 2022 as shown in Figure 4 and Table 2. Results followed this pattern for insect biomass sampled from cut vegetation only (Figure 5), with Frankenmarkt having a higher median insect biomass than Micheldorf (Table 2). Again, differences were not of statistical significance (Mann-Whitney U test = 410; n = 30 plots; $p = 0.559$). However, comparing the insect biomass sampled through ground vacuuming only (Figure 6), Frankenmarkt and Micheldorf varied significantly in July (Mann-Whitney U test = 182; n = 30 plots; $p < 0.001$) with the former having a median lower by a factor of six (19 mg vs. 121 mg) than the latter (Table 2).

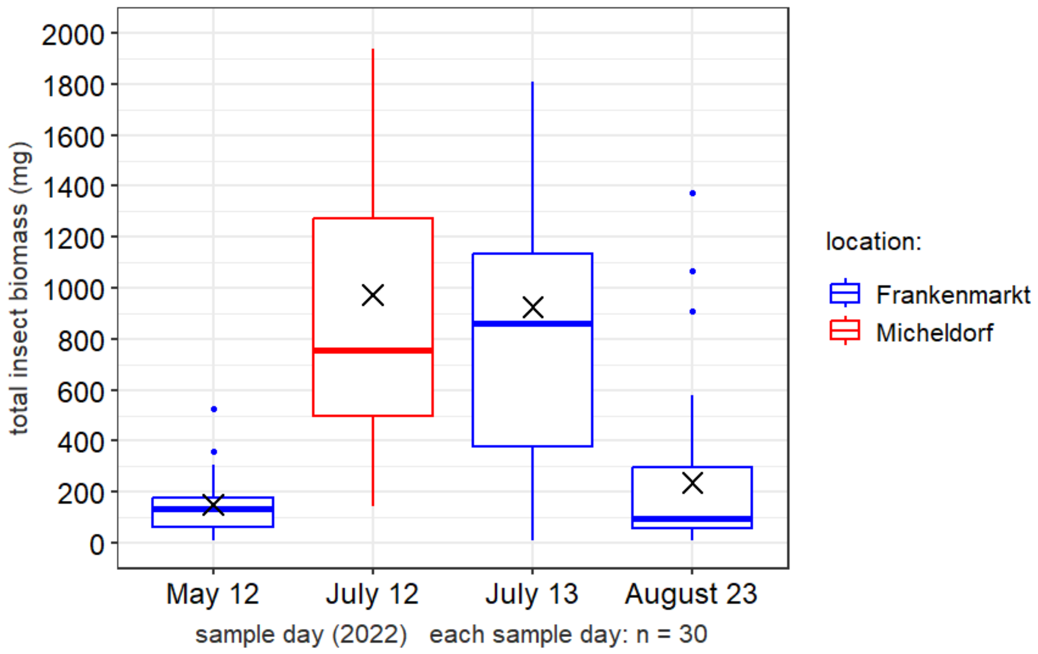

Figure 4. Difference of total insect biomass between extensively and intensively managed meadows in July did not reach statistical significance. The data for Frankenmarkt is represented by the colour blue and includes three days of sampling, while the data for Micheldorf is depicted in red and comprises one sample day. Six outliers are not shown in this graph due to the chosen y-axis scale. Each day of mowing a total of 30 plots were sampled therefore n = 30. For a comprehensive understanding of “n”, mowing techniques and depiction of boxplots, see figure caption of Figure 3.

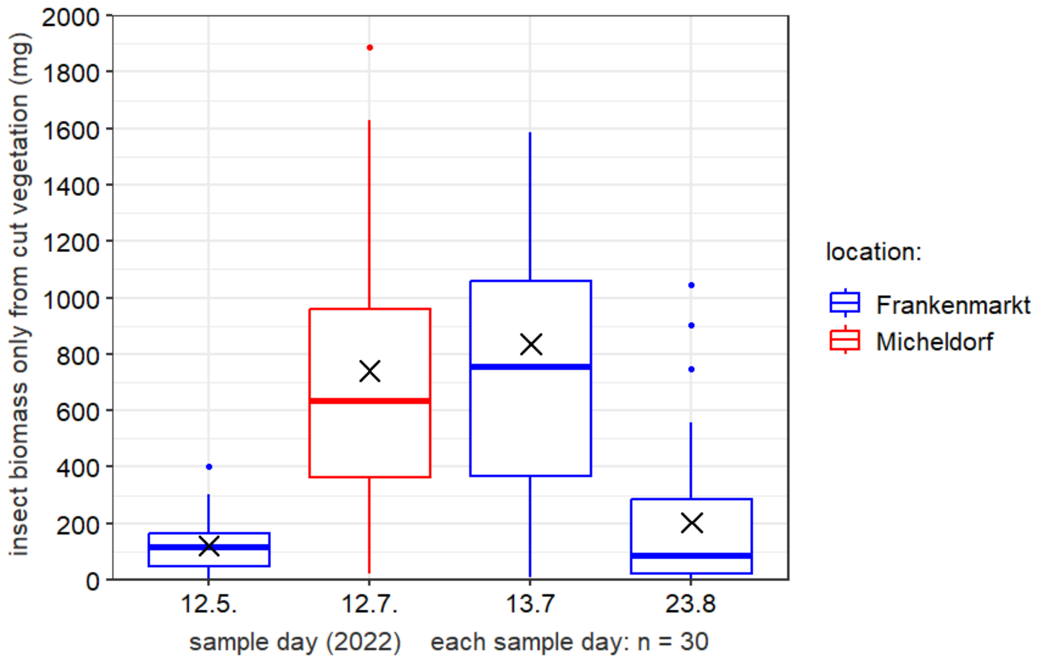

Figure 5. Difference of insect biomass only from cut vegetation between extensively and intensively managed meadows in July did not reach statistical significance. The data presentation corresponds to that of Figure 4. Three outliers are not shown in this graph due to the chosen y-axis scale. Each day of mowing a total of 30 plots were sampled therefore n = 30. For a comprehensive understanding of “n”, mowing techniques and depiction of boxplots, see figure caption of Figure 3.

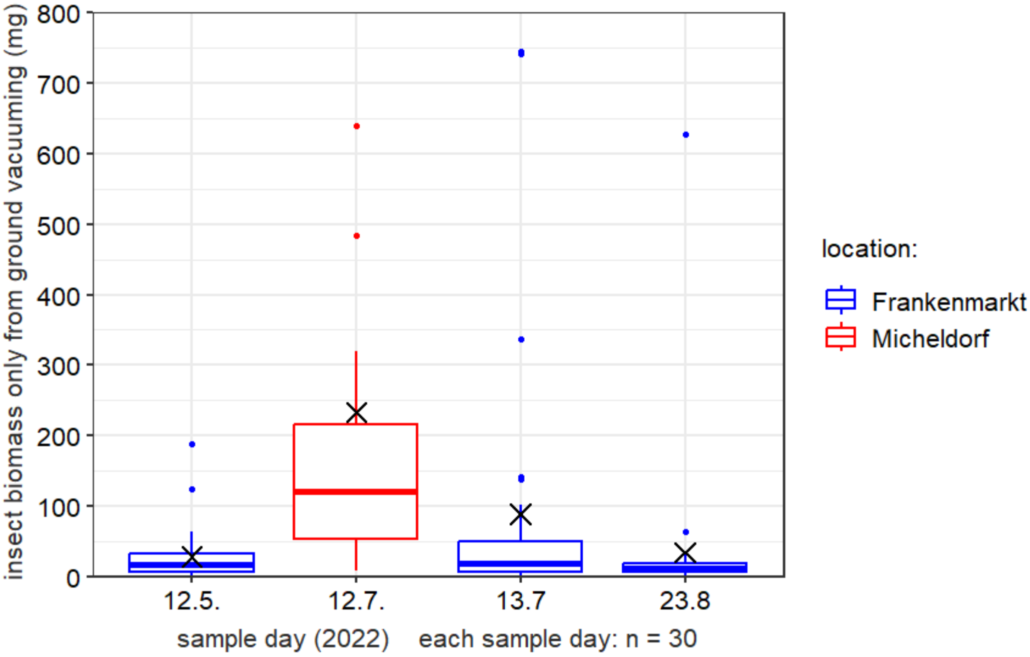

Figure 6. A statistically significant difference in insect biomass collected from the vacuumed ground surface was found when comparing the extensively and intensively managed meadows in July. The data presentation corresponds to that of Figure 4. Two outliers are not shown in this graph due to the chosen y-axis scale. Each day of mowing a total of 30 plots were sampled therefore n = 30. For a comprehensive understanding of “n”, mowing techniques and depiction of boxplots, see figure caption of Figure 3.

Table 3. Insect biomass (mg) varied greatly between sample days and collection methods. Description of collection methods is given in chapter 2.2.1. The table is divided into three sections: 1. Total insect biomass of all plots and sampling methods per sample day. 2. Insect biomass solely from cut vegetation matter per sample day. 3. Insect biomass collected from the vacuumed ground surface per sample day. The median, minimum, maximum, first quartile (Q1), and third quartile (Q3) values, expressed as percentages, are provided for each collection method and sample date. The sample size for each technique was consistent, with 30 plots sampled per collection method. The statistical test, Matt-Whitney U, is included at the bottom of each section to assess the significance of the observed differences between July dates. For a comprehensive understanding of "n", see figure caption of Figure 3.

Total insect biomass								
Date (n = 30 each)	**Location**	**median (mg)**	**min (mg)**	**max (mg)**	**1Q (mg)**	**3Q (mg)**	**sum (mg)**
May 12, 2022	Frankenmarkt	136	12	528	57	180	4509
July 12, 2022	Micheldorf	755	142	2891	482	1297	29223
July 13, 2022	Frankenmarkt	860	11	3101	351	1200	27774
August 23, 2022	Frankenmarkt	97	8	1375	54	318	7125
Statistical test: Mann-Whitney U = 433; p = 0.807; n = 120 (30 plots each day)							
Insect biomass in cut vegetation							
Date (n = 30 each)	**Location**	**median (mg)**	**min (mg)**	**max (mg)**	**1Q (mg)**	**3Q (mg)**	**sum (mg)**
May 12, 2022	Frankenmarkt	118	2	404	37	167	3655
July 12, 2022	Micheldorf	635	23	2413	352	1006	22212
July 13, 2022	Frankenmarkt	755	9	3076	338	1070	25108
August 23, 2022	Frankenmarkt	85	1	1048	21	308	6076
Statistical test: Mann-Whitney U = 410; p = 0.559; n = 120 (30 plots each day)							
Insect biomass vacuumed from the ground							
Date (n = 30 each)	**Location**	**median (mg)**	**min (mg)**	**max (mg)**	**1Q (mg)**	**3Q (mg)**	**sum (mg)**
May 12, 2022	Frankenmarkt	17	2	188	6	34	854
July 12, 2022	Micheldorf	121	9	2065	52	219	7010
July 13, 2022	Frankenmarkt	19	1	746	6	52	2666
August 23, 2022	Frankenmarkt	12	2	627	7	19	1050
Statistical test: Mann-Whitney U = 182; p < 0.001; n = 120 (30 plots each day)							

3.3 Orthoptera: Species composition and abundance differences between meadows

Both meadow sites varied significantly in their number of Orthoptera individuals and species detected in July with Frankenmarkt on July 13, 2022, having approximately 65% less individuals (38 vs. 104) and 60% less species (4 vs. 10) than Micheldorf on July 12, 2022. All detected species of the respective meadow sites and sample dates are given in Table 3. Estimated sum of orthopteran biomass out of all sample vials (cut vegetation "alive" or "dead" and vacuumed ground "alive" or "dead") in Frankenmarkt on July 13, was a third of the biomass in Micheldorf on July 12, 2022 as depicted in Figure 7. Furthermore, as illustrated in Figure 8 and Table 4, orthopterans in the intensively managed meadow in Frankenmarkt were on average approximately 3 mm larger than in the extensively managed meadow in Micheldorf.

Three of the four Orthoptera species detected in July were found again on August 23, 2022 on the intensively managed meadow in Frankenmarkt. A total of 21 individuals were sampled in August. Estimated total biomass was ≈ 1,9 g and median body length of all measured individuals was 10 mm, therefore both values were lower than in July (Table 4)

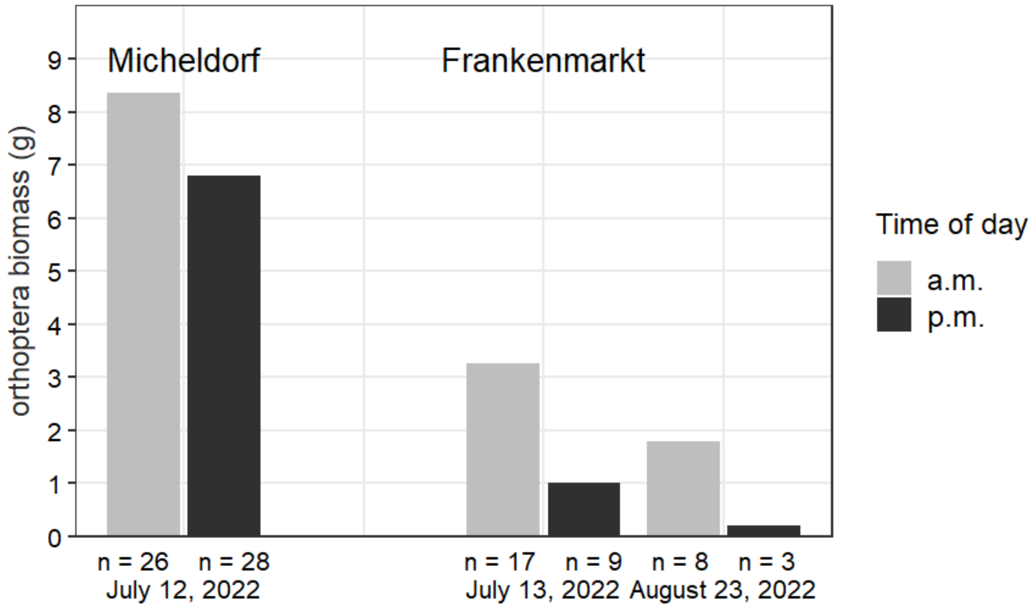

Figure 7. Sum of all total orthopteran biomass per time of day and sample day was signficiantly higher on the extensive meadow in Micheldorf. The x-axis represents the dates of the sample days seperated into morning sampling (a.m.) depicted in light grey and early afternoon sampling (p.m.) depicted in dark grey. The y-axis displays the orthopteran biomass in grams (g). The value "n" represents the sample size i.e, sample vials containing Orthoptera components or individuals. The maximum number of vials per bar is limited to 60 as sampling resulted in four vials per plot (see chapter 2.2.1).

Table 4. The number of Orthoptera species observed per sample date and site. The extensively managed meadow in Micheldorf had the highest species diversity. The last column displays the total count of species per day and site.

July 12, 2022	**July 13, 2022**	**August 23, 2022**
Micheldorf	**Frankenmarkt**	**Frankenmarkt**
-	*Chorthippus albomarginatus*	-
Chorthippus biguttulus	-	-
Chorthippus dorsatus	-	-
Gryllotalpa gryllotalpa	-	-
Gryllus campestris	-	-
Mecostethus parapleurus	-	-
Pholidoptera griseoaptera	-	-
Pseudchorthippus parallelus	*Pseudchorthippus parallelus*	*Pseudchorthippus parallelus*
Roeseliana roeselii	*Roeseliana roeselii*	*Roeseliana roeselii*
Tetrix subulata	*Tetrix subulata*	*Tetrix subulata*
Tettigonia cantans	-	-
Number of species: 10	**4**	**3**

Table 5. Average body length of Orthoptera individuals was largest from individuals from Micheldorf. The median, minimum, maximum, first quartile (Q1), and third quartile (Q3) values, expressed as millimetres, are provided for each sample date. The value "n" represents the sample size i.e, number of sampled and measured intact Orthoptera individuals.

Orthoptera lengths								
Date	**Location**	**n**	**median [mm]**	**arith. mean [mm]**	**min [mm]**	**max [mm]**	**1Q [mm]**	**3Q [mm]**
July 12, 2022	Micheldorf	86	17,00	16,05	10,25	21,00	3,00	34,00
July 13, 2022	Frankenmarkt	32	14,00	13,31	5,75	19,25	4,00	26,00
August 23, 2022	Frankenmarkt	21	10,00	12,43	9,00	12,00	7,00	29,00

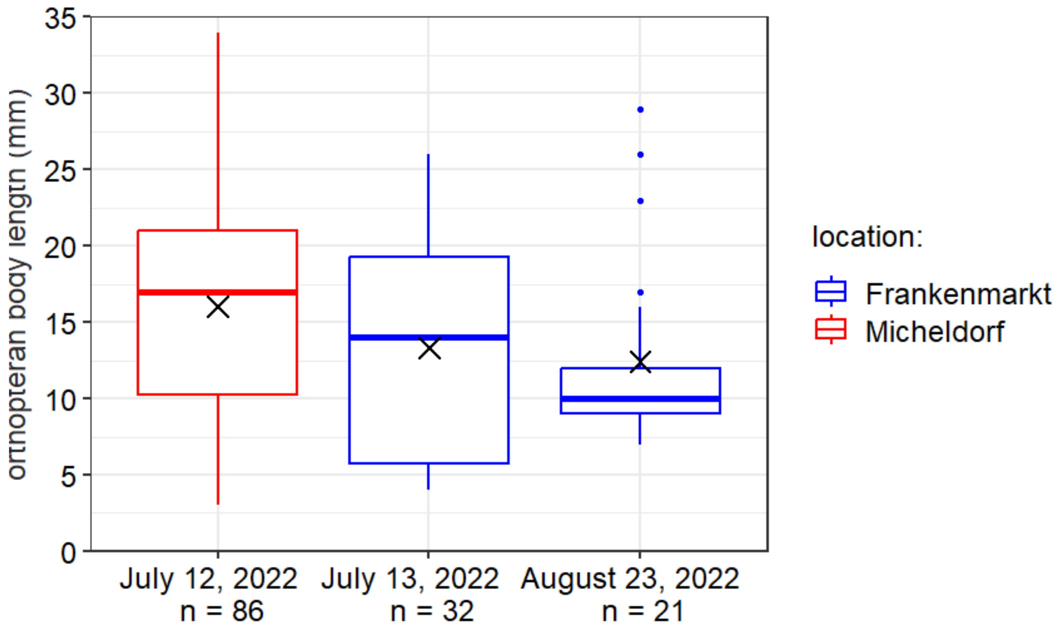

Figure 8. Orthoptera individuals from the extensively managed meadow in Micheldorf were on average slightly larger than in Frankenmarkt. The x-axis represents the dates of observation, while the y-axis represents the orthopteran body length in millimeters. The data for Frankenmarkt is represented by the colour blue and includes two days of sampling, while the data for Micheldorf is depicted in red and comprises one sample day. The value "n" represents the sample size i.e, number of sampled and measured intact Orthoptera individuals. For a comprehensive understanding on the depiction of boxplots, see figure caption of Figure 3.

3.4 Impacts mowing at different time of day the scale of direct insect mortality?

Even though sum and arithmetic mean of dead insect biomass sampled in Micheldorf on July 12, 2022, were lower at mowing in the morning (a.m.) than at early afternoon mowing (p.m.), median from a.m. sampling was approximately 1.5 times higher (Figure 9 and Table 5). In comparison to dead insect biomass sampled in Frankenmarkt July 13, 2022, at a.m. where sum, mean and median were all higher than at p.m.. A lower calculated median of dead insect biomass at p.m. was also evident for both other sample days in Frankenmarkt on May 12, 2022 and on August 23, 2022.

Comparison of total dead insect biomass between all plots sampled in the morning and afternoon (Figure 10 and Table 5) revealed the same pattern of results as for individual sampling days (Figure 9 and Table 5). Differences between obtained a.m. and p.m. biomass did not reach a statistical α significance level of 0.05 (Kruskal-Wallis H test = 1.514; df = 1; n = 120 plots total; p = 0.219).

Therefore, although differences in dead insect biomass for mowing in the morning and early afternoon for the respective dates were not of statistical significance, results indicate a trend. As the mean biomass of dead insect biomass for all plots and sample days was consistently slightly lower in the afternoon, the results provide a basic indication that mowing in the afternoon leads to an overall lower mortality of all occurring meadow insects.

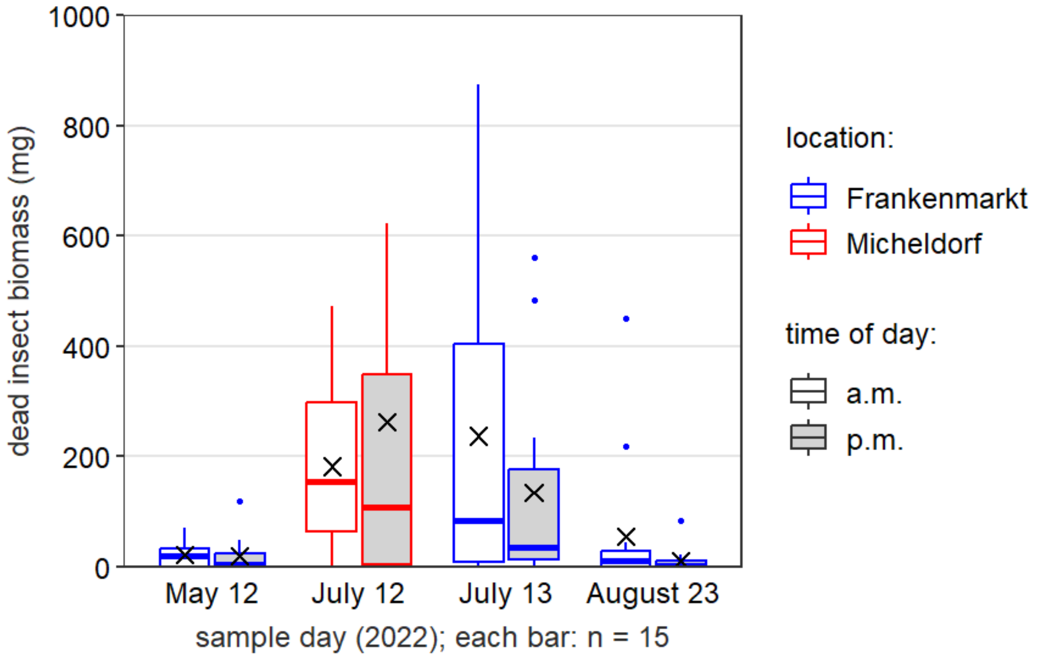

Figure 9. No statistically significant difference between mowing in the morning or early afternoon on the mortality of insects for each sampling day was found. However, results indicate a trend towards lower mortality in the afternoon. The x-axis represents the four different sample days in the year 2022. The data for Frankenmarkt is represented by the colour blue and includes three days of sampling, while the data for Micheldorf is depicted in red and comprises one sample day. Each sample day is represented by two bars, one for the morning sampling (white) and one for the early afternoon sampling (light grey). The associated boxplots show the distribution of dead insect biomass for the respective half-day. The y-axis represents the dead insect biomass in milligram. 1 outlier is not shown in this graph due to the chosen y-axis scale: July 12, p.m.: 1599 mg. "n" represents the sample size i.e., sampled plots for each time of day. Each day of mowing 30 plots were sampled. 15 in the morning and 15 in the early afternoon. Thus, each bar has a sample size n = 15 plots. For a comprehensive understanding on the depiction of boxplots, see figure caption of Figure 3.

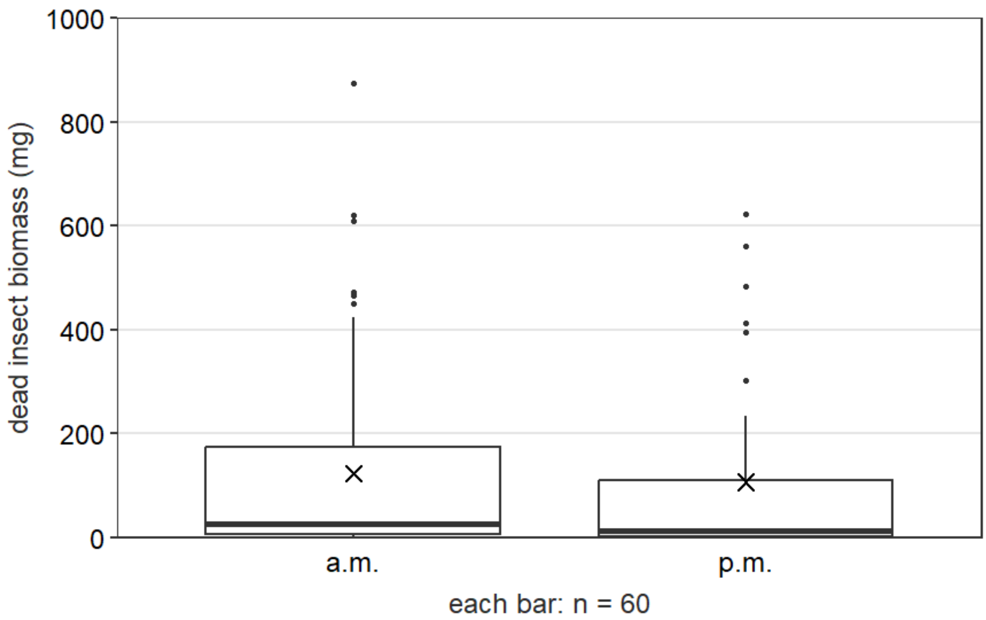

Figure 10. No statistically significant difference between biomass of dead insects of all plots mown in the morning or early afternoon was found. However, results indicate a trend towards lower mortality in the afternoon. The x-axis represents time of day of mowing (a.m. or p.m.). The associated boxplots show the distribution of dead insect biomass for the respective time of day. The y-axis represents the dead insect biomass in milligram. n" represents the sample size i.e., sampled plots for each time of day. A total of 120 plots was sampled, therefore 60 in the morning and 60 in the early afternoon. Thus, each bar has a sample size n = 60 plots. For a comprehensive understanding on the depiction of boxplots, see figure caption of Figure 3.

Table 6. Median of dead insect biomass, therefore mortality through mowing, is lower in the afternoon than at morning. The table is divided into two sections: 1. Biomass of dead insects of all plots per time of day. 2. Biomass of dead insects per time of day, sample day and location. The median, minimum, maximum, first quartile (Q1), third quartile (Q3) values, and sum, expressed in miligram, are provided for each time of day and sample day. The sample size for each time of day 15 plots. The statistical test, Kruskal-Wallis H, is included at the bottom of the first section to assess the significance of the observed differences between a.m. and p.m. dead insect biomass.

Dead insect biomass a.m. vs p.m.: Total									
Date & Daytime (n = 60 each)		**Location**	**median (mg)**	**arith. mean (mg)**	**min (mg)**	**max (mg)**	**1Q (mg)**	**3Q (mg)**	**sum (mg)**
all days	a.m.	all plots	25	124	0	875	5	184	7426
all days	p.m.	all plots	12	107	0	1599	2	116	6394
Statistical test: Kruskal-Wallis H test = 1.514; df = 1; p = 0.219; n = 120 plots total (60 plots a.m./p.m.)									
Dead insect biomass a.m. vs p.m.: For each location and sample date									
Date & Daytime (n = 15 each)		**Location**	**median (mg)**	**arith. mean (mg)**	**min (mg)**	**max (mg)**	**1Q (mg)**	**3Q (mg)**	**sum (mg)**
May 12, 2022	a.m.	Frankenmarkt	20	22	0	69	0	39	324
May 12, 2022	p.m.	Frankenmarkt	3	19	0	119	1	31	287
July 12, 2022	a.m.	Micheldorf	154	182	0	472	24	324	2730
July 12, 2022	p.m.	Micheldorf	107	263	0	1599	3	395	3949
July 13, 2022	a.m.	Frankenmarkt	84	237	0	875	9	466	3555
July 13, 2022	p.m.	Frankenmarkt	34	134	0	561	12	193	2004
August 23, 2022	a.m.	Frankenmarkt	11	54	0	450	0	41	817
August 23, 2022	p.m.	Frankenmarkt	2	10	0	85	0	11	154

3.5 Error rates

The biomass of previously overseen insects i.e., insects only found through re-examination in the laboratory of "emptied in the field" collection bags, was placed in relation to the total biomass of collected insects per corresponding plot, time of sampling and whether if obtained from cut vegetation or vacuumed ground surface. The resulting percentage, representing the error rate of overseen insect biomass, was < 0.01 % in 95 of a total of 173 re-examined collection bag and less than 10,00% in almost all cases. (Figure 11). In five instances, the biomass of the insects found during the detailed re-examination of collection bags in the laboratory, exceeded that biomass determined for insect found in the field. In this cases, single large insect individuals were overseen and outweighed the biomass of numerous smaller insects previously found resulting in exceptionally high calculated error rate (≈ 105 %, ≈ 124 %, ≈ 220 %, ≈ 224 %, ≈ 522 %). Regarding the five tested different mowing techniques, there is an evenly distributed pattern, with error rates of < 0.01 % in most cases for each technique. 42 collection bags were re-examined for DCB, 41 collection bags for RDC and 30 collection bags each for RD, RDC1 and RDC2 (Figure 12).

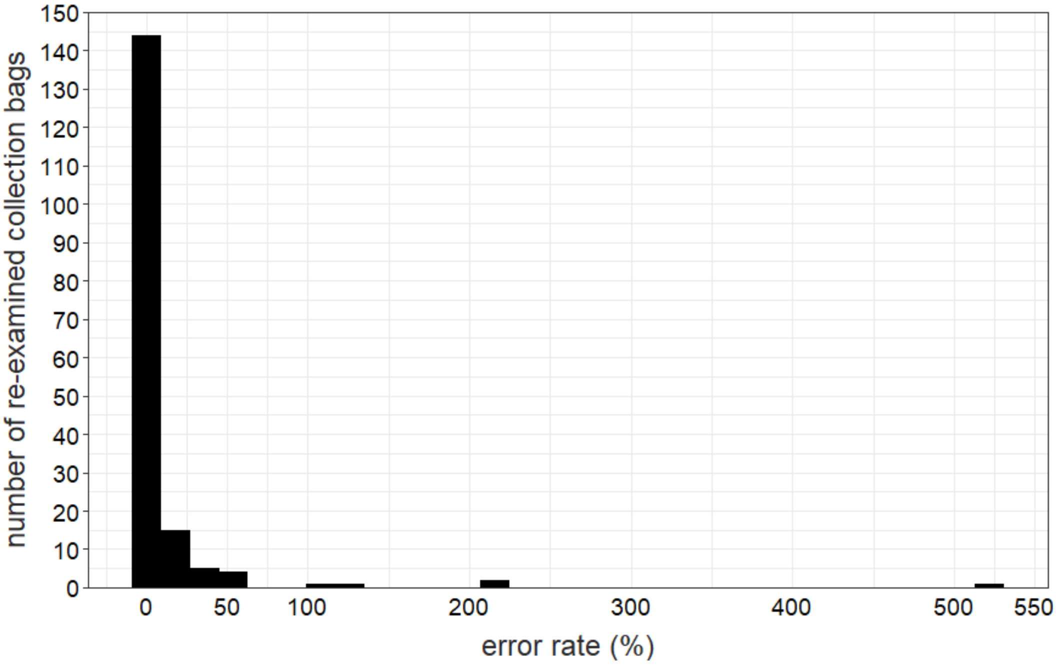

Figure 11. The error rate, representing the calculated proportion of biomass from insects found through re-examining collection bags to biomass from field-detected insects, was in most cases < 0.01%. The x-axis represents the error rate while the y-axis represents the number of re-examined collection bags. Out of 173 bags, the error rate was < 0.01% in 95 bags and < 10.00% in almost all cases. However, the oversight of single large insect individuals such as caterpillars, which outweighed the biomass of numerous smaller insects previously detected, resulted in five exceptionally high calculated error rates (≈ 105%, ≈ 124%, ≈ 220%, ≈ 224%, ≈ 522%).

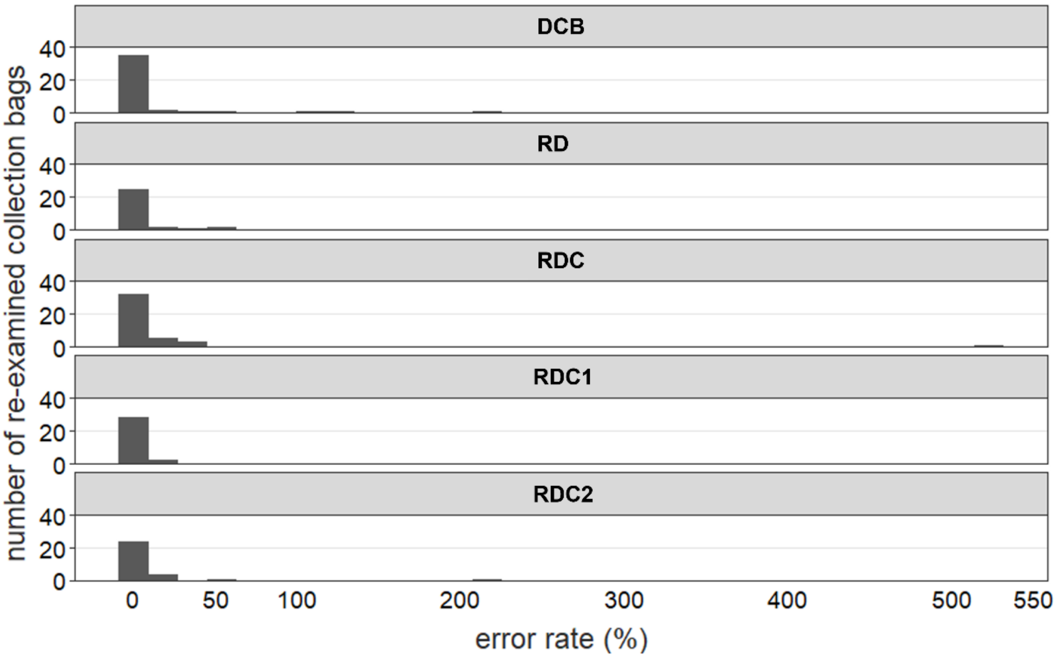

Figure 12. The error rates for each mowing technique, representing the calculated proportion of biomass from insects found through re-examining collection bags to biomass from field-detected insects, were evenly distributed and in most cases < 0.01%. 42 collection bags were re-examined for DCB, 41 collection bags for RDC and 30 collection bags each for RD, RDC1 and RDC2. The x-axis represents the error rates while the y-axis represents the number of re-examined collection bags. For a comprehensive understanding on the calculated error rates, see figure caption of Figure 11.

4 Discussion

4.1 Five different mowing techniques

This study investigated for potential differences in the accumulated biomass of severely damaged or dead insects due to the use of different mowing techniques. Results confirm that physical damage on insects by the cutting machinery varies greatly depending on the utilized mowing technique.

The tested double bar mower (DCB) has the least impact, therefore producing the smallest proportion of severely damaged or killed insect biomass to total insect biomass with a median of 0.58 %. Detected proportion of damaged/killed insect biomass to surviving insect biomass, which is equivalent to insect mortality, is higher when using a rotary disc mower (RD). This finding, regarding DCB having lower general insect mortality compared to RD follows previous observations by other authors, whereby the latter`s mortality can be twice as high (Blodgett et al., 1995; Humbert et al., 2009, 2010; Stöckli et al., 2020). Mortality of insects i.e., proportion of damaged or killed insect biomass to total insect biomass, increases substantially with an overall mean between 20 - 30 % by applying a conditioner, even when the conditioner is additionally equipped with a harrow or deflective plate. These results are in line with previous studies as well, as shown by Humbert et al. (2010) where rotary mowers with conditioners had a insect mortality three to four times higher compared to a double blade bar mower.

While this study conducted a holistic approach which comprised all observed insects larger than three millimetres, disregarding their taxonomy, the vast majority of previously conducted studies focused on specific insect taxa. Even though such study designs may produce contradictory results depending on the focal taxonomic group (Mackey & Currie, 2001; Bruggisser et al., 2010; Pedley & Dolman, 2014; Proske et al., 2022), findings comparing the mortality impact of double blade bar mowers and rotary disc mowers without and with conditioner are consistent for various insect taxa and correspond with the present study`s results.

Focusing solely on Orthoptera, a double blade bar mower led to 9 % injured or killed grasshoppers whereas numbers increased three times as much if a rotary drum mower, a technique not utilized in this study, with or without a conditioner was utilized and peaked at 37% impact by using a rotary disc mower with conditioner (Oppermann et al., 2000; Oppermann, 2007). Similar results were obtained by Wilke (1992), who also took the cutting height in account, estimating an impact rate of 5,6% for a double blade mower at 6 cm height, in contrast to a rotary disc mower 29,7 % at the same height, respectively 30.4 % at a cutting height of 3 cm. However, it must be pointed out that, a low number of studied individuals and only two replicants seriously limit the robustness of this study as stated before by Humbert (2010).

Studies on the mortality of beetles (Coleoptera) due to physical damage caused by mowing equipment also tested in this present study (cutter bar mower and rotary disc mower with our without conditioner) are barely conducted yet and if, impact is regarded as being very low (Cizek et al., 2012). Nevertheless Blodgett et al. (1995) identified a sickle bar mower as their tested method with the least impact. Notably, results obtained by Frick and Fluri (2001) seem to suggest that even insects considered as highly mobile are affected by the mowing machinery. The percentage of severely injured or killed honeybees was 5 % out of the extrapolated number of observed individuals prior to the mowing if a rotary drum mower was used. Attaching a conditioner resulted in a drastic increase of affected bees with 35 %.

The general findings on direct insect damage by different mowing techniques of this present study are in line with previous research, such as Humber et al. (2010). Not only that a bar mower has less impact i.e., least amount of severely damaged/killed insect biomass, compared to rotary mower but also that the utilization of a conditioner increases dead insect biomass significantly (Humber et al., 2010). Physical damage is a direct result of the mechanical crimping and crushing of the cut vegetation by the conditioner, resulting in severely injured or killed insects (Frick & Fluri, 2011). If the goal of such a large-scale, industrialised mowing is to improve the survival rate of insects in concerning meadows, these results, supported by previous studies, indicate that the use of a double blade bar mower favours insect survival rate compared to a rotary disc mower. In addition, simply not using a conditioner to reduce direct insect mortality is a very effective and comparatively easy to implement measure for ensuring this goal.

4.2 Differences between the extensively and intensively managed site

Insect and spider diversity is well known to be negatively affected by an intensifying mowing regime, due to the restricted timeframes of only a couple of weeks for development and regeneration between mowing passes, alongside the loss of individuals by the mowing equipment (Cizek et al., 2013; Unterweger et al., 2017; Feng et al., 2021; Wersebeckmann et al., 2021). Especially arthropods associated with upper strata of the meadow vegetation, such as folivorous Orthoptera, orb-weaver spiders or flower-visitors like many Hymenoptera or Lepidoptera are particularly affected by the mowing machinery (Blodgett et al., 1995). On the other hand, Blodgett et al. (1995) postulate that invertebrates, which live mainly on the ground such as Carabidae or Staphylinidae, are hardly affected by the mowing process. Thus, it was hypothesized in this present study that the diversity and biomass of insects in the intensively mowed meadow, especially in the cut vegetation only, would be significantly lower when compared to the extensively managed meadow.

Wintergerst et al. (2021) compared the diversity of multiple arthropod taxa and the overall arthropod biomass between nine meadows which were partially mown (10 – 30 % of the meadow left uncut) twice annually at most and nine intensively mown sites that were fully mown two to five times per year. The mean arthropod biomass (1.26 – 4.51 g vs. 0.01 – 0.33 g) as well as mean number of identified species (13.6 – 24.8 species vs. 0 – 3 species) per sampling were both significantly higher (Mann–Whitney U, $p < 0.01$, $n = 9$) on the meadows with lower mowing frequency and preserved parts left uncut, compared to meadows with a high mowing frequency. Mean number of species observed as per sampling was also significantly higher (Mann–Whitney U, $p < 0.01$, $n = 9$) on extensively managed meadows than on intensively managed ones (2.4 - 8.6 species vs. 0 – 0.4 species). An observation also supported by Humbert et al (2010) who suspected animals with limited mobility to be especially vulnerable to mowing. Thus, Wintergerst et al. (2021) propose the implementation of reduced mowing frequencies alongside partial mowing regimes as viable strategies to enhance the diversity, abundance, and reproductive success of insects in grassland habitats such as urban lawns, agricultural meadows, and conservation zones.

The importance of unmown meadow parts as a sheltering refugium for invertebrates was also validated by Cizek et al. (2012). Behavioural study of lepidoptera revealed active avoidance of uniformly cut meadows and preference for uncut meadow strips or blocks. Abundance of studied ground beetle, orthopteran and spider was higher in unmown parts. The authors determined that simultaneous mowing of large meadow areas leads to the sudden loss of available essential resources such as food or shelter, the unification of habitat structures and a large-scale direct mortality resulting in the loss of arthropod abundance as well as diversity.

No statistically significant differences in the total insect biomass, as well as insect biomass only from cut vegetation, between the intensively and extensively managed meadow were observed in this present study. Surprisingly, the intensive meadow in Frankenmarkt showed even higher biomass, albeit not significantly. Therefore, these results are contrary with those of the Wintergerst et al. (2021) study, which employed a comparable methodology.

A limitation of this present study is that, due to time constraints paired with substantial methodological effort, only two sites were examined and compared regarding insect biomass, while Wintergerst et al. (2021) encompassed 18 sites. Furthermore, the sampled number of herbivorous Lepidoptera and “Symphyta” caterpillars in July, which significantly contribute to biomass, was significantly higher in Frankenmarkt (171 individuals) as compared to Micheldorf (25 individuals). The predominant part of all caterpillars found in Frankenmarkt belonged to only two Lepidoptera species, namely *Autographa* cf. *gamma* and *Xestia c-nigrum* (pers. comment: Jonathan Schwarz). One explanation for the absence of biomass differences between the two meadows is, that some generalist and widespread species, such as formerly mentioned Lepidoptera species, are highly successful on the intensive meadow and attain high population densities. Despite lower observed biodiversity of occurring plant species in the intensively managed meadow, similar or even marginally higher biomass levels of insects are achieved. This is supported by Haddad et al. (2000, 2001) and Klink et al., (2020) who demonstrated that long-term nitrogen addition (fertilization) decreases insect and plant species richness but increases number of insect individuals and plant productivity. Concurrently, also total insect biomass and abundance of herbivores and detritivores were positive correlated with nitrogen addition, profiting from the increases. This indicates that fertilization over an extended period results in simplified communities of plant and insect species yet supports biomass and abundance of a few selected species. (Siemann et al., 1998; Haddad et al., 2000)

The findings of this present study reveal contrasting results itself, regarding the insect biomass differences between both meadow sites, when solely examining the insect biomass from the vacuumed ground. Hereby the biomass of the extensive meadow is significantly higher than that of the intensive meadow which is contrary to the differences from looking solely at insect biomass from cut vegetation. This is primarily due to the considerable number of ant individuals in the samples from Micheldorf. Based on theoretical reasoning by author, one potential explanation for this is, that ants, especially those constructing their nests above ground, have more time to establish greater colonies if mowing intervals are longer, thus leading to increased abundance and biomass. Ants are recognized for their significant role as general predators, controlling co-occurring invertebrate populations (Hölldobler & Wilson, 1990; Lach et al., 2010; Cerdá & Dejean, 2011). Therefore, a speculative suggestion by the author based on Hölldobler & Wilson (1990) as well as Seifert et al. (2016) is, that ant regulation prohibits extremely high population density of only a few invertebrate species in the extensive meadow, while still comparable biomass is achieved through the diversity of species and respective individuals.

4.3 Orthoptera occurrence and body length differences

During the sampling of this study ten species of Orthoptera were confirmed in the extensively managed meadow in Micheldorf (MD), which was only mown once in July 2022. On the intensively managed meadow in Frankenmarkt (FM), which was mown four times the same, only four species of grasshopper were detected through sampling. Along with the number of species, also the number of individuals was significantly higher on the extensively managed meadow compared to the intensively managed one. This pattern of results is consistent with available similar findings such as Hiller & Betz (2014) who investigated for variance in Orthoptera populations on adjacent parts of several meadows due to different mowing frequencies. Sampling sites with a monthly mowing interval had, with the exception of one out of six meadows, no grasshoppers or katydids present at all. Contrary to that, parts of the meadows only mown once per year and not earlier than July had up to eight different species. The negative impact of frequent and especially early mowing on orthopteran abundance was also studied by Buri et al. (2013). Their results provide evidence that the numbers of individuals increase up to fivefold if the first mowing is set to the middle of July. While mowing is often followed by a sharp decline in abundance of grasshoppers and katydids (Rada et al., 2014), their relatively high mobility allows for fast recolonization from habitats which are under less mowing pressure (Chisté et al., 2016). Besides the direct physically damage due to the mowing equipment, an increased risk of predation, the removal of eggs with cut vegetation, and overheating or desiccation of the eggs due to a lack of protection of surrounding plant matter are the greatest threats to Orthoptera associated with mowing (Gardiner and Hassall 2009; Humbert et al., 2010).

All four species of Orthoptera that were sampled in FM are widespread generalists of open grassland habitats in Austria and frequently found in intensely mown and humid meadow habitats (Gardiner et al., 2002; Landmann, 2016; Kenyeres & Szentirmai, 2017; Zuna-Kratky et al., 2017). This finding is in direct line with Gardiner (2006), who also detected three (*C. albomarginatus, P. parallelus* and *R. roeselii*) of the four species found in FM on intensively managed pastures in England. The fourth species detected in FM is *Tetrix subulata*, which was also one of the most abundant species on a productive meadow mown twice a year in Check Republic (Cizek et al., 2012). It is important to point out, that one individual of *Mecostethus parapleurus* and two individuals of *Gryllus campestris* have been observed, although not sampled, in FM as well. Thus, at least six species are present in FM. However, it is presumed that the population sizes of those species, observed outside the designated periods and sample sites according to the methodology, are very low or that they were immigrant individuals from surrounding habitats.

The extensively managed meadow in MD is characterized by various Orthoptera species which are associated with humid or mesophilic conditions. *Chorthippus dorsatus*, for example, is characterizes by achieving the highest abundance in wet and extensively managed meadows and quickly vanishes with an increasing frequency of fertilizing and mowing (Detzel, 1998; Landmann, 2016; Szanyi et al., 2021). Other species found in MD are also described as commonly found in wet or humid habitats are *Gryllotalpa gryllotalpa, Tettigonia cantans* or *Tetrix subulata*. (Gavlas et al., 2007; Flügel, 2010). Several individuals of *M. parapleurus*, a species occurring mainly in wet biotopes and bound to an extensive mowing regime (Landmann, 2016), were sampled in MD, indicating a high abundance. The habitats surrounding the study site in MD consist of one alder carr (*Alnus glutinosa*), several wet meadows and artificial ponds (Bejvl, 2010). It is assumed that these conditions, additionally promoted by an extensive mowing regime, are the reason for the variety of hygrophilous Orthoptera species occuring in MD.

One aspect of this study was to investigate if average body length of Orthoptera is correlated with the frequency of mowing events. The results clearly support this, with a smaller mean body length verified for the meadow mown four times in FM. In addition to average length of all detected individuals per site, more species that generally grow larger have also been recorded in MD, such as *Mecostethus prapleurus* or *Tettigonia cantans*. Contrary to that, a rather small species *Tetrix subulata* was found at all sample dates in FM and MD. This is also in accordance with Oppermann et al. (2000) who linked the extent of the mowing impact also to body size among other factors such as cutting height. In their study more than 30 % of individuals larger than 20 mm were injured, while Orthopter smaller than 11 mm were almost never affected. Other researchers have obtained similar results and shown that larger species such as *Metrioptera bicolor*, *Stethophyma grossum* or *Leptophyes punctatissima* tend to be more susceptible to mowing injuries (Oppermann & Krismann, 2001; Wagner, 2004; Gardiner & Hill, 2006; Zuna-Kratky et al., 2017). Although, there is clear evidence that larger individuals and species of Orthoptera face greater exposure to physical damage from mowing than smaller ones, it must me pointed out that all developmental stages and sizes are fatally affected by the mowing process (Gardiner & Hill, 2006). These are important findings for understanding how an intensive mowing frequency has potential negative effects on orthopterans and how such management practices can alter the existing species composition.

4.4 What is the impact of mowing time of day on the scale of insect mortality?

This survey investigated for a general tendency of the impact of mowing time (morning vs. early afternoon) on insect mortality. The results could not show any statistical significance between morning and afternoon mowing regarding the sampled biomass of dead insects. Nevertheless, it must be highlighted, that the same pattern is discernible for all mowing days which indicates a slightly lower insect mortality in the afternoon. It is hypothesised that one possible explanation for this observation could be that, as temperatures rise during daytime, insect activity generally increases, which allows them to better avoid the mowing machinery. Only a limited number of previous studies focused on a potential optimal daytime of mowing for insects and findings regarding this question are contradictory (Van der Poel & Zehm, 2015).

For example, in a well replicated study with over 30 plots Oppermann et al. (2000) identified solely the type of mowing equipment utilised as a significant factor for differences in insect mortality.

Other investigated factors such as time of mowing, temperature or wind strength were not identified to have any effect.

The outcome of above-mentioned study differs from Dover et al. (2010) and Cizek et al. (2012) who argue that highly mobile species like Lepidoptera face greater damage through mowing carried out early in the morning i.e., with lower temperatures, due to increased inactivity. According to these results, the damaging impact, at least on butterflies, can be reduced by mowing at midday when temperature and therefore activity and capability to escape is increased.

Contrary to above findings, are recommendations for wildlife and insect-friendly mowing from the Deutsche Wildtier Stiftung [eng: "German wildlife foundation "] abbreviated as DWS (2005). With the intention to protect flower-visitors, thus potentially pollinators, mowing should be carried out when their activity is low. Therefore, preferably early in the morning, late in the evening or when weather is cool and clouded. This statement is supported by the findings of Fluri et al. (2000), who found that the number of honeybee individuals in the meadow increased with clear weather and rising air temperature. The authors recommend mowing in the early morning or in the evening when foraging activity of *Apis mellifera* is lowest. Although these results are in accordance with the suggestions from DWS, they are limited by solely focusing on flying, pollinating insects, disregarding all other meadow inhabitants. Furthermore, it must be pointed out, that honeybees are only impacted by mowing machinery if they are foraging, in contrast to other affected species, which resident permanently in the habitat. Therefore, scheduling the daily time of mowing based on honeybee activity seems not to be advisable if the aim is to reduce the overall impact on all occurring insects.

This study chose a universal approach by determining biomass of all sampled dead insects, to minimize the bias of looking at specific taxa, thus potentially misjudging the impact of mowing time. However, it suffers from the limitation that only time of day was examined as a comparator without considering differences in weather or temperature between morning and afternoon mowing. Therefore, to determine a potential optimal time of day for mowing regarding minimising overall insect mortality, future research should be carried out to provide possible insight.

4.5 Quantification of error rates

The present study aimed to assess the occurring error rate, regarding overseen insect individuals while sampling with proposed method to establish a reproducible and robust evaluation method for future studies. To achieve this goal, the biomass of previously overseen insects was compared to the total biomass of collected insects per corresponding plot and time of sampling.

Insect biomass has widely been used as a measurement tool for assessment studies on insect population sizes and their dynamics (Macgregor et al., 2019, Schuch et al., 2019; Svenningsen et al., 2022; Fürst et al., 2023; McCluney et al., 2023) particularly following results from Hallmann et al. (2017), which reported a dramatic decrease in insect flying biomass within German nature reserves. Because small bodied species out-number large body species in a habitat, the potential of biomass underestimating abundance rates must be taken in consideration (Hallmann et al., 2020; Guo et al., 2020). Nevertheless, a significant and high correlation between biomass and abundance i.e., number of individuals, has been proven for various taxa of insects (e.g. Schuch et al., 2019; Hallmann et al., 2020; van Klink et al., 2020; Vereecken et al., 2021). This is not the case for biomass and species richness (Hallmann et al., 2020; Vereecken et al., 2021). Consequently, number of individuals and biomass are equally suitable tools for similar analyses of insect communities (Schuch et al., 2023).

The results of this study showed that the error rate of overseen insect biomass was in almost all cases very low (less than or equal to 10 % in 145 of 173 counts). The low and evenly distributed error rates observed for all the individual mowing techniques, indicate a high degree of robustness in the tested approach. To establish robust evaluation methods, it's necessary to identify reliable parameters. Therefore, insect biomass, instead of relying on the number of individuals, can be a practical and accessible classification parameter, even without extensive taxonomic knowledge or time-consuming counting of individual insects. Developing evaluation methods that incorporate weighing of biomass can lead to easily implementable assessments.

Nonetheless, it must be pointed out, that the very low error rate quantified in the present study was directly reliant on the accuracy of the researchers present in the field, conducting the sorting of insect. Therefore, the extent of detection bias will vary across different studies. The potential impact of such differences on the interpretation of results remains to be studied in future investigations. Furthermore, different observers may have varying level of expertise which can influence the identification and classification of insect fragments or individuals. Detection bias can easily occur if insects are not identified as such or overlooked while the collection bags are examined. The use of magnifying glasses or stereomicroscopes can aid in accurate identification. As shown in the present study, careful re-examination can mitigate or at least quantify this detection bias.

4.6 Conclusion

In conclusion, the present study provides valuable insights regarding the direct mortality of insects due to different mowing techniques in agricultural grasslands. The utilized type of mowing equipment was detected as a significant impact factor on the extent of direct insect mortality during mowing passes. Although the biomass difference of dead insects between morning and afternoon mowing was not statistically significant, a consistent trend of slightly lower insect mortality in the afternoon erose. This suggests that mowing during this time of day might be favourable for mitigating negative impact on insects. Additionally, this study proposes the quantification of insect biomass as a possible evaluation method for monitoring and comparing the effects of different mowing techniques on insect communities.

Further research is necessary to deepen our understanding of other mowing-associated factors on invertebrates such as weather conditions, avoidance mechanism of various taxa, the impact of the vehicles on ground dwelling organism or impact of mowing speed. Exploring these additional research topics will help to identify potential interactions and confounding factors and therefore to provide a more nuanced understanding of the complex relationships between mowing and insect diversity. This, in turn, could facilitate the development of more effective conservation strategies to mitigate the negative impacts of existing agricultural practices on insect populations and ensure the conservation of present insect biodiversity.

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