

Common garden versus common practice – Phenotypic changes in *Trifolium pratense* L. in response to repeated mowing

Thomas Gross^{*#}, Christina Magdalena Müller[#], Annette Becker, Birgit Gemeinholzer, Volker Wissemann

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Summary

Plants react to various biotic and abiotic factors by adjusting their growth pattern. This process, called phenotypic plasticity, can also be observed in the agronomical important fodder plant *Trifolium pratense* L. (red clover) which is grown worldwide for its high protein content and soil improving ability. After cutting it changes its growth pattern due to phenotypic plasticity and initiates a second growth phase. Here, we analyze the regrowth dynamics of red clover in response to grazing or mowing by recording the plant's architecture, leaf morphology, and growth performance in different cutting experiments. As previous studies were limited in the number of individuals and carried out via common garden experiments, we analyzed the regrowth reaction of *T. pratense* under standard agricultural field conditions. *T. pratense* forms smaller and rounder leaflets after repeated cutting, compensated by the production of more biomass. Nitrogen contents were subjected to seasonal changes, rather than by changes through cutting. As middle to late cut plants had higher regrowth capacities and regrew more biomass than early cut plants, an optimal time point for biomass harvest can be suggested to maximize the total yield of red clover biomass.

Keywords: red clover, mowing, phenotypic plasticity, leaf roundness, biomass production

Introduction

Plants are exposed to various biotic and abiotic factors, which they cannot evade due to their sessile nature. Therefore, they adapt their growth behaviour to local environmental constraints leading to phenotypic variation owing to their open blueprint (DOMAGALSKA and LEYSER, 2011; TEICHMANN and MUHR, 2015; GASTAL and LEMAIRE, 2015; PRINS and VERKAAR, 1992). Detailed phenotypic plasticity responses due to environmental perturbations were observed in many different plant species like *Trifolium repens* L., *Populus trichocarpa* Hook., and *Pisum sativum* L. (STAFSTROM, 1995; GOULAS et al., 2002; RYLE et al., 1985; WU and STETTLER, 1998). A major factor inducing phenotypic plasticity is the loss of biomass through herbivory or cutting. The Fabaceae *T. repens* reacts to cutting by producing more but smaller leaves compared to uncut plants (GOULAS et al., 2002). As *T. repens* is a common fodder plant this effect might be of agronomical importance, as repeated cutting could reduce the amount of harvested biomass (GASTAL and LEMAIRE, 2015; PRINS and VERKAAR, 1992; BRISKE and RICHARDS, 1995).

A close relative to *T. repens*, *Trifolium pratense* L. (red clover), is used in many parts of the world as high-quality fodder plant due to its naturally high protein content, deep root systems, and high yield which give *T. pratense* preference over *T. repens*. It is grown in monocultures or in a grass mixture where it increases the nitrogen

content of the surrounding plants (ERIKSEN et al., 2012; FERNANDEZ and WAREMBOURG, 1987; BEECHER et al., 2015; DEWHURST, 2013; KLEEN et al., 2011). In addition, red clover produces high amounts of the enzyme polyphenol peroxidase which increases both nitrogen uptake and polyunsaturated fatty acids by livestock (LEE et al., 2006; WINTERS and MINCHIN, 2005) and reviewed in VAN RANST et al. (2011). Furthermore, digestibility analyses have shown the advantages of re-grown red clover biomass in terms of protein content and digestible fiber content. In addition to varying growing conditions throughout the season, the cutting regime of red clover has a great influence on the harvested biomass, with respect to number of cutting events and their time points (WIERSMA et al., 1998). However, both factors are also dependent on the respective red clover cultivar and their perseverance (MARSHALL et al., 2017; WIERSMA et al., 1998).

In a previous study, phenotypic plasticity of red clover during regrowth after cutting/mowing was analysed in a common garden experiment (HERBERT et al., 2018). After cutting, red clover produces, similar to *T. repens*, more leaves than uncut plants but they are of the same size than before cutting. Only the length of newly formed petioles is reduced when compared to uncut plants. Taken together, cutting triggers a second growth phase in red clover and could thereby contribute to yield increase. However, this analysis was performed under artificial conditions as a common garden experiment in a greenhouse using only a limited number of plants and these were cut only once. We were interested in the growth changes of *T. pratense* grown under field conditions with multiple cutting events and how they differ from the changes observed in the common garden. In particular, it is interesting to see whether the second growth phase after the cut also contributes to increased yields in field trials.

Here, we investigated the native response of locally adapted wild red clover plants as used in HERBERT et al. (2018) to cutting and how different mowing regimes influence leaf morphology.

We hypothesized that field grown red clover plants will only partially reflect the reaction shown by the common garden greenhouse experiment from HERBERT et al. (2018) as they are more prone to abiotic and biotic stresses. Thus, the questions arise (i) whether and how plants compensate cutting losses during regrowth and by this if the experiment of HERBERT et al. (2018) can be validated under common practice conditions in a field experiment, (ii) if different mowing regimes influence the amount of harvested biomass of the natural population red clover, and (iii) whether nitrogen content, as a marker for protein content and fodder quality, differs in the leaflets after mowing.

Material and methods

The experimental fields were located at the Agricultural Research and Developmental Farm of the Justus-Liebig-University Giessen in Rauischholzhausen/Germany (50°45'55.9008"N, 8°52'1.2648"E). The red clover seeds are derived from the same wild populations in Saxony, Saxony-Anhalt, Thuringia, Thuringian-Forest, and Ucker-

* Corresponding author

These authors contributed equally to this work

marck as described in HERBERT et al. (2018), (Rieger Hofmann seed company; Blaufelden, Germany). We sowed the red clover in autumn 2016, the mowing started in 2017. The plots were 2 m wide and 3 m long. Twelve different mowing regimes (Tab. 1), with 3 plots per regime, were applied. For further analysis plots 1, 3, 4, 6, 7, 9, 10, and 12 were considered, especially plots 4, 7, and 10 as those were mown three times. Each mowing regime was carried out three times on randomly distributed plots (Fig. 1, Tab. 1). The measurements were taken 18-20 days after mowing to evade the first stress responses. For each treatment, plant height, number of inflorescences, length and width of five leaflets per plant of ten randomly chosen plants were measured from three plots, totalling 30 plants per treatment and 150 leaflets. To avoid boundary effects in morphological measurements only plants internal of a 50 cm strip around the edges of each tested plot were analysed. The following characters were recorded: weight of the Fresh Material (FM), height of the plants, width and length of one leaflet, from the latter two characters we determined the roundness and the leaflet area. To calculate the roundness we used leaflet length/leaflet width, the leaflet area was determined by $(\text{leaf length}/2) * (\text{leaf width}/2) * \pi$. For nitrogen content measurements, the harvested *T. pratense* material was dried after mowing at 90 °C. The nitrogen content was measured with CN analyser Vario MAX (Elementar Analysensysteme GmbH, Hanau, Germany) (data was uploaded to the BExIS database and is accessible under <https://doi.org/10.25829/bexis.26586-5>, <https://doi.org/10.25829/bexis.26606-1>, and <https://doi.org/10.25829/bexis.26666-1>). For the questioning and the statistical calculation, we grouped the different plots in classes together after the time of mowing (Tab. 1): early means the time between 12.6-12.09, middle: 03.07-02.10, late 14.07-13.10 and later 31.07-17.10, regardless of time: all plot's which are mown three times (4, 7, and 10). Plot 1 is the unmown control field. The statistical tests and graphics were conducted in R (R CORE TEAM, 2013) with the following packages PMCMR (POHLERT, 2014) and ggplot2 (WICKHAM, 2009). We tested for normality with the Shapiro-Wilk test and vari-

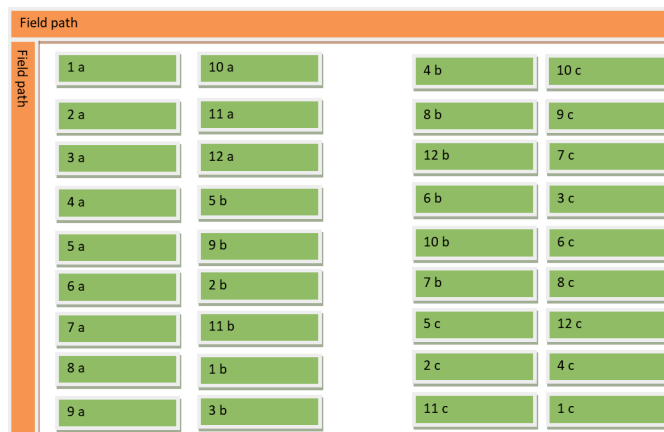


Fig. 1: Structure of the test field of *Trifolium pratense*. 1-12 the different mowing regimes, a-c the randomly distributed plots.

ance homogeneity was tested with Bartlett test, if both tests were true, we applied a one-way analysis of variance (ANOVA). The Tukey HSD post hoc test was used to identify significant ANOVA results. If the normality and/or the variance homogeneity was not true, the Kruskal-Wallis test was applied and the Posthoc-Kruskal-Dunn test to further analyse the significant results of the Kruskal-Wallis test. Additionally we tested 16 different generalized linear mixed models (GLMMs) using Template Model Builder (TMB) (BROOKS et al., 2017), for the impact of Classes (early, middle, late, and later) the Cut systems (1/2/3/no) and the Time in the year (Supplement Tab. 1). The final model was chosen by the Akaike information criterion (AIC; Supplement Tab. 1). After first model validation, we transformed the data for leaflet roundness (standardized) and leaflet area (log) (Supplement Fig. 1, 2 and 3).

Tab. 1: Mowing regime and dates of measurement for each plot

Plot	classes	mowing regime	1 st mowing	Date of measurement	2 nd mowing	Date of measurement	3 rd mowing	Date of measurement
1 (a-c)		not mown		every time		every time		every time
2 (a-c)	early	1× mown	middle of June	30.06.2017				
3 (a-c)	early	2× mown	middle of June	30.06.2017	middle of August	29.08.2017		
4 (a-c)	early	3× mown	middle of June	30.06.2017	middle of August	29.08.2017	middle of September	03.10.2017
5 (a-c)	middle	1× mown	beginning of July	20.07.2017				
6 (a-c)	middle	2× mown	beginning of July	20.07.2017	end of August	19.09.2017		
7 (a-c)	middle	3× mown	beginning of July	20.07.2017	end of August	19.09.2017	beginning of October	17.10.2017
8 (a-c)	late	1× mown	middle of July	31.07.2017				
9 (a-c)	late	2× mown	middle of July	31.07.2017	middle of September	03.10.2017		
10 (a-c)	late	3× mown	middle of July	31.07.2017	middle of September	03.10.2017	middle of October	31.10.2017
11 (a-c)	later	1× mown	end of July	18.08.2017				
12 (a-c)	later	2× mown	end of July	18.08.2017	beginning of October	17.10.2017		

Results and discussion

For plant height and the number of inflorescences, we could show that with increasing mowing frequencies the plant height and the number of inflorescences decreased compared to the not mown control group (Suppl. Fig. 4 A and B). While these results show nicely the cutting effect on the regrowth ability of the red clover plants, our investigation concentrated on leaflet roundness and the potential increase in leaflet area associated with mass build-up in response to repeated cutting. For this, we identified the effect of different mowing frequencies (no cut, 1. cut, 2. cut, 3. cut) and time of mowing in the year (classes: early, middle, late, later) on leaflet roundness, leaflet area and the amount of harvested biomass.

For all plots, which were mown at least two times (3, 4, 6, 7, 9, 10, and 12), the roundness of leaflets increased after the 2nd cut $P < 0.001$ (no normality distribution and no variance homogeneity $P < 0.001$), compared to the unmown red clover plants (control plants, plot 1) only the 2nd cut had significantly increased leaflet roundness. The 1st cut and the control plants showed no changes in leaflet roundness ($P = 0.82$; Suppl. Fig. 5 A).

For the roundness of leaflets after three cuts regardless of the classes in the plots 4, 7, and 10 the data are not normally distributed ($P = 0.003$) and there is no variance homogeneity ($P = 0.018$; Fig. 2 A), the plots within the different cuts were compared. The Kruskal-Wallis test detected significance in the roundness of leaflets ($P < 0.001$) after cutting and the Posthoc-Kruskal-Dunn test identified that there are significant differences between all three cuts with $P < 0.001$. In the three plots 4, 7 and 10, differences were detected between plot 4 and the two other plots (7 and 10) in leaflet roundness ($P < 0.001$; Fig. 2 A). Compared to the control group (plot 1) there are no significant differences between the plots in the 1st cut and the control group ($P = 0.129$) in leaflet roundness. After the 1st cut, the roundness of the leaflets varied between 0.4 - 0.6, 2th cut 0.57-0.77 and 3rd cut 0.57-0.87 (with 1 being round; Fig. 2 A). The leaflet roundness increased with

each cutting. Especially the plots 7 and 10 showed a great increase after the 2nd and 3rd cut ($P = 0.01$, Fig. 2 A and 3 B, C).

Considering the four mowing classes (early, middle, late, and later), we observed a significant ($P < 0.001$) increase in leaflet roundness in all of our classes, regardless of the time period of mowing (Fig. 3 A-D). For plot 4 the roundness of leaflets after mowing differed significantly ($P < 0.001$) from the plots 7 and 10 (Fig. 2 A). We detected a significant difference in the roundness of leaflets in plot 4 between the 1st/2nd ($P = 0.002$) and 1st/3rd ($P < 0.001$) mowing (Fig. 3 A). Between the 2nd and 3rd mowing there were no differences detectable in Plot 4 ($P = 0.49$; Fig. 3 A).

Our model (LM5) and the data show that the production of rounder leaflets after mowing is enhanced through multiple mowing events (Cut and Date; $\chi^2 < 0.001$, $P < 0.001$) and independent of the season. As HERBERT et al. (2018) mowed red clover only once, this increase in roundness was not detectable. Leaf shape is often subjected to phenotypic plasticity depending on nutrient or water supply, and on temperature (LI et al., 2019; DORKEN and BARRETT, 2004; ROYER et al., 2009). However, leaflet roundness increases regardless of the classes, indicating a low or no contribution of these abiotic factors to the change in roundness. Interestingly, leaf shape is also dependent on the age of the plant, and in *Glycine max* (L.) Merr. older plants form less round leaflets (YOSHIKAWA et al., 2013). Hence, we can propose that cutting rejuvenates *T. pratense* and early regrowth produces juvenile, rounder, leaflets.

The leaflet area did not change for plots mown twice (3, 4, 6, 7, 9, 10, and 12) $P = 0.160$ (no normality distribution and no variance homogeneity $P < 0.001$) and only compared to the control plants the 1st and 2nd cut showed a significant decrease in the leaflet area ($P < 0.001$; Suppl. Fig. 1 B). After three cuts, regardless of the classes in the plots 4, 7, and 10, the leaflet area data is not normally distributed ($P < 0.001$) and there is no variance homogeneity ($P = 0.026$; Fig. 2 B). Significance was detected by the Kruskal-Wallis test in the leaf-

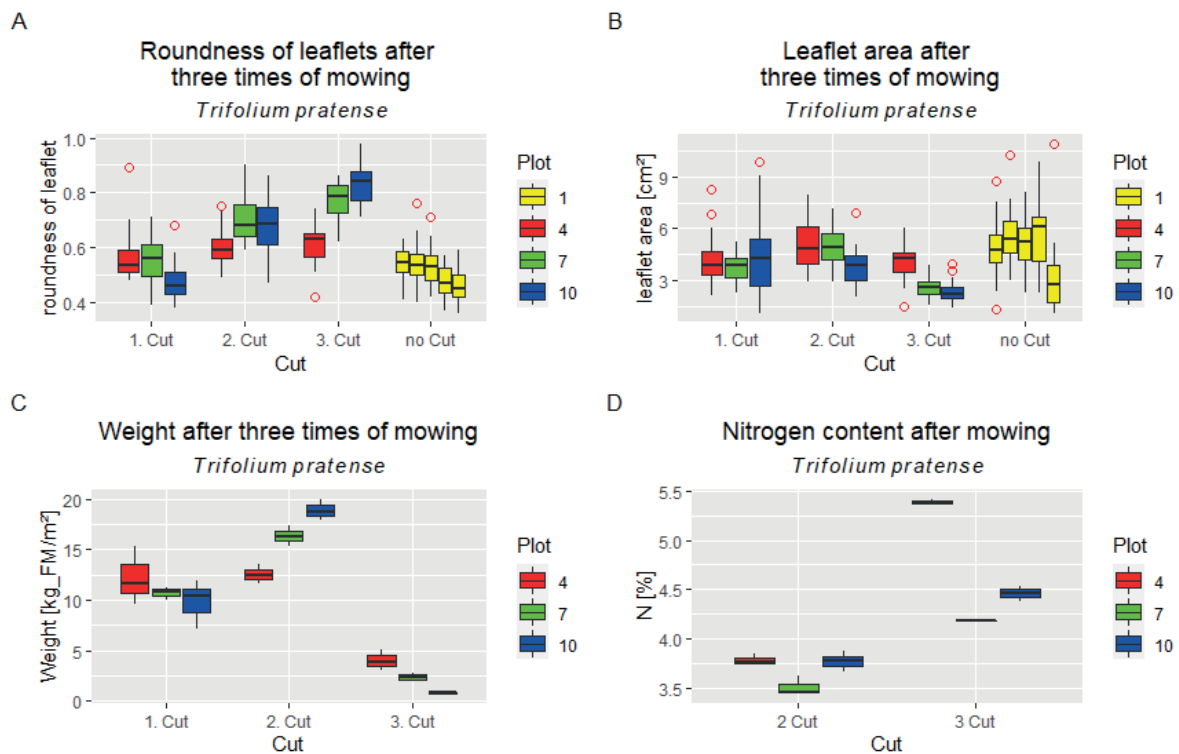


Fig. 2: Development of (A) roundness of leaflets after mowing three times (up to 1 for round), (B) leaflet area [cm²] after mowing three times, (C) weight [kg_FM/m²] of the harvested plots after mowing three times in the year regardless of the season and (D) the nitrogen content [%] in the leaves after mowing. Red dots = outlier; Plot: 1 = not mown, 4 = early, 7 = middle, 10 = late.

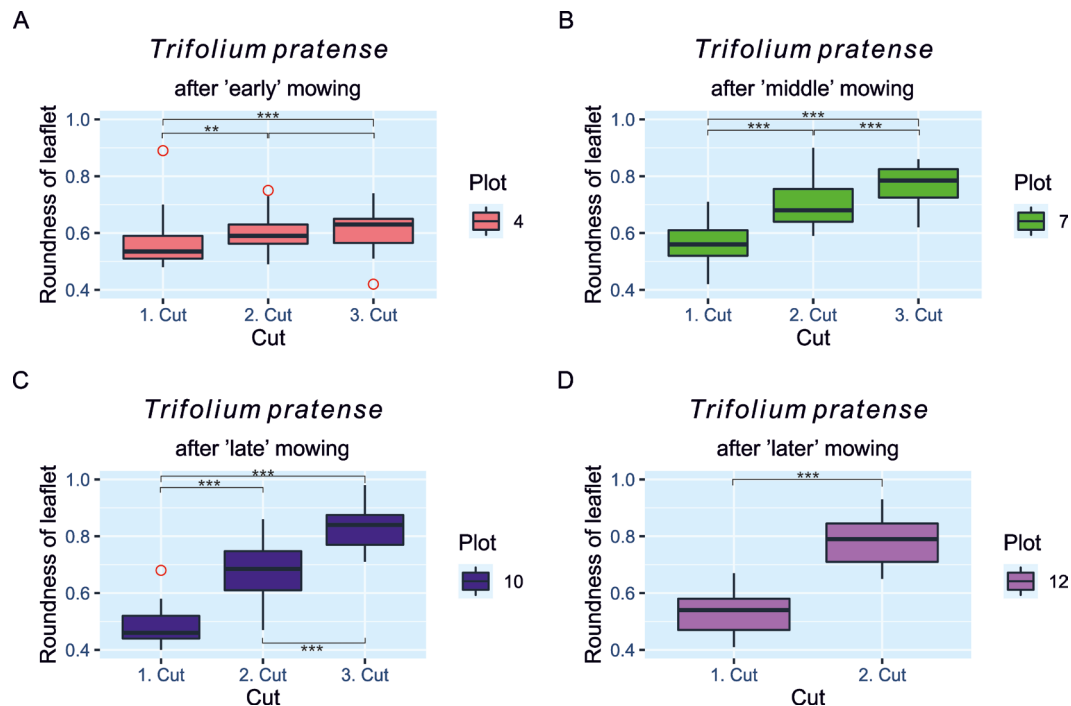


Fig. 3: Development of leaflet roundness (up to 1 for round) (A) after early, (B) middle, (C) late and (D) later mowing in the year. ** $P < 0.01$, *** $P < 0.001$

let area ($P < 0.001$) after cutting and the Posthoc-Kruskal-Dunn test identified significant differences between all three cuts in terms of leaflet area with $P < 0.001$. Differences in leaflet area were detected between plot 4 and the two other plots (7 and 10, $P < 0.001$; Fig. 2 B). Compared to the control group (plot 1) there are no significant differences between no cut and 2nd cut ($P = 0.399$). After first mowing, the leaflet area varied between 4-5 cm² and remained stable after the second mowing (Fig. 2 B). Only after the third cut, the leaflet area of *T. pratense* decreases (Fig. 2 B). The Model LM4 shows a significant influence of mowing frequencies ($\chi^2 < 0.001$, $P < 0.001$) and the time of mowing ($\chi^2 < 0.001$, $P < 0.001$) for leaflet area.

Considering the four treatment classes (early, middle, late, and later), we observed an increase of leaflet area after the 2nd cut and a decrease after the 3rd cut in which it returns to the size of the 1st cut (early $P < 0.001$) or below (middle, late, later $P < 0.001$; Fig. 2 B).

For plot 4 the leaflet area after mowing differed significantly ($P < 0.001$) from the plots 7 and 10 between the second and third mowing. The leaflet area in plots 7 and 10 decreased slightly after the 3rd cut (Fig. 2B), the leaflet area in plot 4 is relatively stable during the three times of mowing. Thus, the leaflet area of *T. pratense* remains rather stable even after two mowing events. This is in contrast to the reaction described in HERBERT et al. (2018) and in *T. repens* (GOULAS et al., 2002), in which the leaflet area decreased already after the first cut. Red clover shows greater regrowth capabilities under field conditions than in common garden greenhouse conditions.

For all plots mown twice (3, 4, 6, 7, 9, 10, and 12), we observed a significant increase in weight with 46.02 % ($P < 0.001$) (Suppl. Fig. 5 C). The biomass weight over three cuts regardless of the season was normally distributed ($P = 0.083$) and variance homogeneity was detected ($P = 0.206$). An ANOVA showed highly significant weight differences between the biomasses of different mowing regimes and the Tukey HSD post hoc test showed highly significant weight differences among / between all three cuttings (Fig. 2 C) but not among the three plots (4, 7 and 10; $P = 0.997$). After the first cut, 8.18 kg Fresh Material (FM)/m² were harvested in total (Fig. 2 C, Tab. 2). Through the second time of mowing 11.97 kg FM/m² were harvested (Fig. 2 C, Tab. 2), thus the plants produced 46.38 % more above ground bio-

Tab. 2: Weight development after three times of mowing regardless of the classes (only aspect the three cuts) and dependent on the class (early, middle, late, and later). Weight measure in kg fresh material (FM) per m².

mowing	Plots	weight kg_FM/m ²	difference in weight kg_FM/m ²	%
regardless of time				
1. Cut	4, 7, 10	8.18		
2. Cut	4, 7, 10	11.97	3.79	46.38
3. Cut	4, 7, 10	1.81	-10.16	-84.87
early				
1. Cut	4	3.05		
2. Cut	4	3.14	0.09	3.01
3. Cut	4	1.01	-2.13	-67.90
middle				
1. Cut	7	2.68		
2. Cut	7	4.09	1.42	52.96
3. Cut	7	0.59	-3.50	-85.54
late				
1. Cut	10	2.45		
2. Cut	10	4.73	2.28	93.20
3. Cut	10	0.21	-4.52	-95.56
later				
1. Cut	12	2.38		
2. Cut	12	3.45	1.08	45.26

mass after the first cut, a result similar to observations by ERIKSEN et al. (2012) in a ryegrass/clover mixture. ERIKSEN et al. (2012) also showed that a third cut reduced yield/weight of red clover. These results described here from the field experiments are in concordance with the common garden greenhouse experiments of HERBERT et al. (2018) after which red clover initiates a new growth phase after cutting to compensate the loss of biomass. Similarly, this was also observed for the relative *T. repens*, which compensates the leaf loss

after defoliation in 5-9 days (RYLE et al., 1985; BLACK et al., 2009). However, repeated cutting seems to decrease the regeneration ability of red clover (ERIKSEN et al., 2012). These results are based only on the “three times mowing per year” regime regardless of mowing date. However, analysing the dates of mowing more thoroughly (Tab. 2, Fig. 3), suggests that mowing twice in the middle and late vegetation period is more beneficial for the gain of biomass weight, similar results for different cutting regimes were shown by WIERSMA et al. (1998). An increase of weight of 50% -90% was observed after the second cut, but a reduction in biomass of -84.87% as compared to the first cutting after the third cutting. The later cutting regime (only two cuts) improved yield to 45.26% (Tab. 2). However, it should be noted that there were 60 days between the 1st and 2nd cut and only 30 days between the 2nd and 3rd cut. However, the daily growth rate between the 1st and 2nd cut is 0.02 kg FM/m²/day, 0.073 times higher (early, middle, late) than between the 2nd and 3rd cut, thus we can assume that even with 60 days between the 2nd and 3rd cut, the amount of harvested biomass would have been less than after the 1st cut.

For the early mowing the harvested biomass weight remained the same between the 1st and 2nd cut ($P = 0.921$) and decreased after the 3rd cut, in the other three classes the weight increased after the 1st cut and decreased after the 2nd cut (Fig. 3 A-D). Temporal analysis of plot 4 revealed that an early mowing is less suitable for increasing growth (Fig. 2 C, 3 A), similar results for an early mowing in the season were shown by WIERSMA et al. (1998) which resulted in an reduced yield.

We detected a change in nitrogen content over the year in the different mowing regimes 2nd cut – 3rd cut ($P < 0.001$). In all three plots, the nitrogen content increased during the season. Plot 4 had the biggest increase of the nitrogen content from 3.76% to 5.39% (Fig. 3 D), while the increases in plots 7 and 10 were lower, 3.46% to 4.18% and 3.77% to 4.46%, respectively. Based on the differences between plot 4 to 7 ($P = 0.03$; Fig. 3 D), mowing neither increased nor decreased the nitrogen content in our red clover plants. DAHLIN and MÅRTENSSON (2008) could show a similar effect for *T. repens* after mowing. However, they used potted plants and mowed only once, thus the seasonal effect is missing. Nevertheless, even repeated cutting did not reduce the nitrogen content, such that high protein content and quality of the harvested material remains stable.

The perseverance of red clover was not a primary target of analysis in this study, but we found the absence of cutting negatively affecting the survival of red clover plants. After the eighth month, the red clover had died on the unmown control plots while mown red clover plots were healthy (Suppl. Fig. 6 and <https://doi.org/10.25829/bexis.26586-5>).

Conclusion

In summary, we could validate the findings of the common garden greenhouse experiment from HERBERT et al. (2018) into the field. *T. pratense* compensates the losses after cutting through starting a new growth phase, especially when the cutting was in the middle of June leading to fresh weight gain. Different mowing regimes influence the harvested biomass: mowing at a later time in the year and after two cuts plants produce the highest biomass in the field. However, the timing of the mowing had no influence on the N-content. Mowing early in the season does not significantly increase the biomass but mowing in the middle or later in the vegetation period (beginning of June) increases the biomass of the harvested red clover and by this an increased total nitrogen content was achieved. However, even very late mowing (end of July) is more profitable than mowing three times early in the season (Tab. 2).

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Conflict of interest

No potential conflict of interest was reported by the authors.

References

- BEECHER, M., HENNESSY, D., BOLAND, T.M., MCEVOY, M., O'DONOVAN, M., LEWIS, E., 2015: The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility. *Grass Forage Sci.* 70, 19-29. DOI: [10.1111/gfs.12081](https://doi.org/10.1111/gfs.12081)
- BLACK, A., LAIDLAW, A., MOOT, D., O'KIELY, P., 2009: Comparative growth and management of white and red clovers. *Ir. J. Agric. Food Res.* 48, 149-166.
- BRISKE, D., RICHARDS, J., 1995: Plant responses to defoliation: a physiological, morphological and demographic evaluation. In: Bedunah, D.J., Sosebee, R.E. (eds.), *Physiological ecology and developmental morphology*, 635-710. Denver, Colorado: Society for Range Management.
- BROOKS, M., KRISTENSEN, K., BENTHEM, K., MAGNUSON, A., BERG, C., NIELSEN, A., SKAUG, H., MÄCHLER, M., BOLKER, B., 2017: glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal* 9, 378. DOI: [10.32614/RJ-2017-066](https://doi.org/10.32614/RJ-2017-066)
- DAHLIN, A.S., MÅRTENSSON, A.M., 2008: Cutting regime determines allocation of fixed nitrogen in white clover. *Biol. Fertil. Soils* 45, 199-204. DOI: [10.1007/s00374-008-0328-9](https://doi.org/10.1007/s00374-008-0328-9)
- DEWHURST, R., 2013: Milk production from silage. Comparison of grass, legume and maize silages and their mixtures. *Agric. Food Sci.* 22, 57-69. DOI: [10.23986/afsci.6673](https://doi.org/10.23986/afsci.6673)
- DOMAGALSKA, M.A., LEYSER, O., 2011: Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* 12, 211-221. DOI: [10.1038/nrm3088](https://doi.org/10.1038/nrm3088)
- DORKEN, M.E., BARRETT, S.C.H., 2004: Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *J. Ecol.* 92, 32-44. DOI: [10.1111/j.1365-2745.2004.00857.x](https://doi.org/10.1111/j.1365-2745.2004.00857.x)
- ERIKSEN, J., ASKEGAARD, M., SØEGAARD, K., 2012: Complementary effects of red clover inclusion in ryegrass-white clover swards for grazing and cutting. *Grass Forage Sci.* 69, 241-250. DOI: [10.1111/gfs.12025](https://doi.org/10.1111/gfs.12025)
- FERNANDEZ, M.P., WAREMBOURG, F.R., 1987: Distribution and utilization of assimilated carbon in red clover during the first year of vegetation. *Plant Soil* 97, 131-143. DOI: [10.1007/BF02149831](https://doi.org/10.1007/BF02149831)
- GASTAL, F., LEMAIRE, G., 2015: Defoliation, shoot plasticity, sward structure and herbage utilization in pasture. Review of the Underlying Ecophysiological Processes. *Agriculture* 5, 1146-1171. DOI: [10.3390/agriculture5041146](https://doi.org/10.3390/agriculture5041146)
- GOULAS, E., LE DILY, F., SIMON, J.-C., OURRY, A., 2002: Morphological pattern of development affects the contribution of nitrogen reserves to regrowth of defoliated white clover (*Trifolium repens* L.). *J. Exp. Bot.* 53, 1941-1948. DOI: [10.1093/jxb/erf045](https://doi.org/10.1093/jxb/erf045)
- HERBERT, D.B., EKSCHMITT, K., WISSEMANN, V., BECKER, A., 2018: Cutting reduces variation in biomass production of forage crops and allows low-performers to catch up. A case study of *Trifolium pratense* L. (red clover). *Plant Biol.* 20, 465-473. Stuttgart, Germany. DOI: [10.1111/plb.12695](https://doi.org/10.1111/plb.12695)
- KLEEN, J., TAUBE, F., GIERUS, M., 2011: Agronomic performance and nutritive value of forage legumes in binary mixtures with perennial ryegrass under different defoliation systems. *J. Agric. Sci.* 149, 73-84. DOI: [10.1017/S0021859610000456](https://doi.org/10.1017/S0021859610000456)

- LEE, M.R.F., COLMENERO, J.D.J.O., WINTERS, A.L., SCOLLAN, N.D., MINCHIN, F.R., 2006: Polyphenol oxidase activity in grass and its effect on plant-mediated lipolysis and proteolysis of *Dactylis glomerata* (cocksfoot) in a simulated rumen environment. *J. Sci. Food Agric.* 86, 1503-1511.
- LI, G., HU, S., HOU, H., KIMURA, S., 2019: Heterophylly: Phenotypic plasticity of leaf shape in aquatic and amphibious plants. *Plants* 8. DOI: [10.3390/plants8100420](https://doi.org/10.3390/plants8100420)
- MARSHALL, A.H., COLLINS, R.P., VALE, J., LOWE, M., 2017: Improved persistence of red clover (*Trifolium pratense* L.) increases the protein supplied by red clover/grass swards grown over four harvest years. *Eur. J. Agron.* 89, 38-45. DOI: [10.1016/j.eja.2017.06.006](https://doi.org/10.1016/j.eja.2017.06.006)
- POHLERT, T., 2014: The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R package.
- PRINS, A., VERKAAR, H., 1992: Defoliation: do physiological and morphological responses lead to (over) compensation? In: Ayres, P.G. (ed.), *Pests and Pathogens. Plant responses to foliar attack*, 13-31. Oxford: Bios Scientific Publishers.
- R CORE TEAM, 2013: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- ROYER, D.L., MEYERSON, L.A., ROBERTSON, K.M., ADAMS, J.M., 2009: Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. *PloS one* 4, e7653. DOI: [10.1371/journal.pone.0007653](https://doi.org/10.1371/journal.pone.0007653)
- RYLE, G.J.A., POWELL, C.E., GORDON, A.J., 1985: Defoliation in white clover: regrowth, photosynthesis and N₂ fixation. *Ann. Bot.* 9-18.
- STAFSTROM, J., 1995: Influence of bud position and plant ontogeny on the morphology of branch shoots in pea (*Pisum sativum* L. cv. Alaska). *Ann. Bot.* 76, 343-348. DOI: [10.1006/anbo.1995.1106](https://doi.org/10.1006/anbo.1995.1106)
- TEICHMANN, T., MUHR, M., 2015: Shaping plant architecture. *Front. Plant Sci.* 6, 233. DOI: [10.3389/fpls.2015.00233](https://doi.org/10.3389/fpls.2015.00233)
- VAN RANST, G., LEE, M.R.F., FIEVEZ, V., 2011: Red clover polyphenol oxidase and lipid metabolism. *Animal* 5, 512-521. DOI: [10.1017/S1751731110002028](https://doi.org/10.1017/S1751731110002028)
- WICKHAM, H., 2009: *ggplot2: elegant graphics for data analysis*. New York, Springer-Verlag New York.
- WIERSMA, D.W., SMITH, R.R., SHARPEE, D.K., MLYNAREK, M.J., RAND, R.E., UNDERSANDER, D.J., 1998: Harvest Management Effects on Red Clover Forage Yield, Quality, and Persistence. *J. Prod. Agric.* 11, 309-313. DOI: [10.2134/jpa1998.0309](https://doi.org/10.2134/jpa1998.0309)
- WINTERS, A.L., MINCHIN, F.R., 2005: Modification of the Lowry assay to measure proteins and phenols in covalently bound complexes. *Anal. Biochem.* 346, 43-48. DOI: [10.1016/j.ab.2005.07.041](https://doi.org/10.1016/j.ab.2005.07.041)
- WU, R., STETTLER, R.F., 1998: Quantitative genetics of growth and development in *Populus*. III. Phenotypic plasticity of crown structure and function. *Heredity* 81, 299-310. DOI: [10.1046/j.1365-2540.1998.00397.x](https://doi.org/10.1046/j.1365-2540.1998.00397.x)
- YOSHIKAWA, T., OZAWA, S., SENTOKU, N., ITOH, J.-I., NAGATO, Y., YOKOI, S., 2013: Change of shoot architecture during juvenile-to-adult phase transition in soybean. *Planta* 238, 229-237. DOI: [10.1007/s00425-013-1895-z](https://doi.org/10.1007/s00425-013-1895-z)

ORCID

Thomas Gross  <https://orcid.org/0000-0003-1194-6670>

Christina Magdalena Müller  <https://orcid.org/0000-0003-4632-3166>

Annette Becker  <https://orcid.org/0000-0002-3229-2162>

Birgit Gemeinholzer  <https://orcid.org/0000-0002-9145-9284>


Volker Wissemann  <https://orcid.org/0000-0003-0345-2603>

Address of the corresponding author:

Thomas Gross, Justus Liebig University, Institute of Botany, Heinrich-Buff-Ring 38, 35392 Giessen, Germany

E-mail: Thomas.Gross@bot1.bio.uni-giessen.de

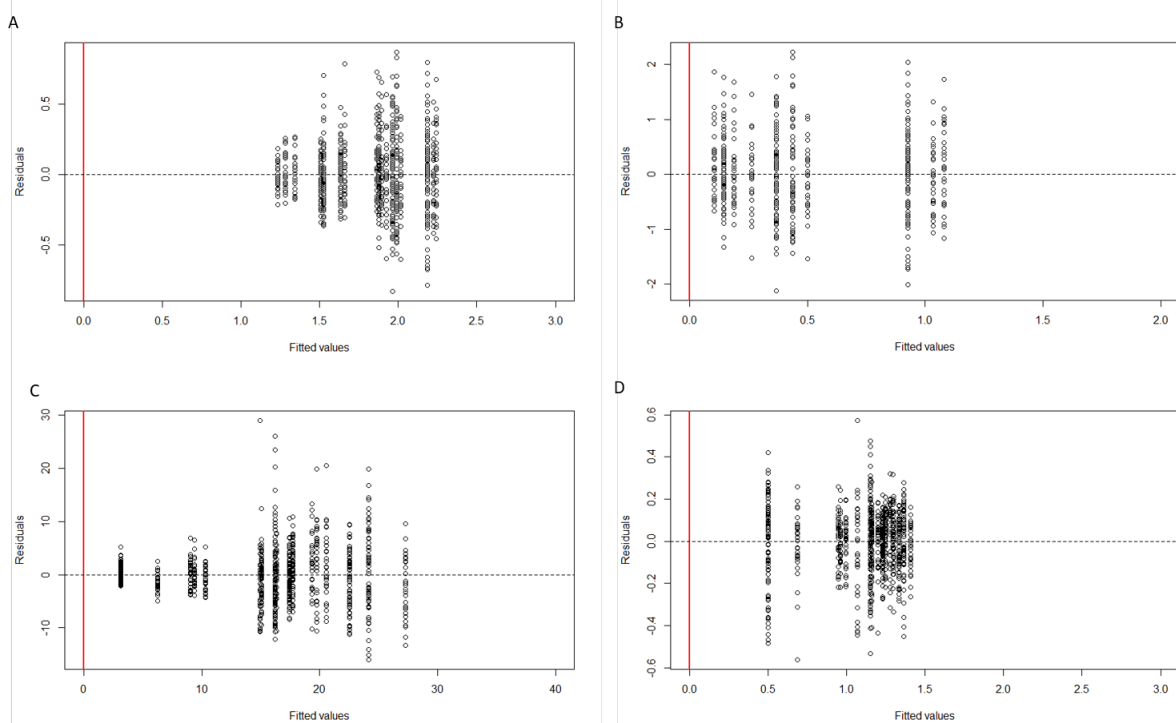
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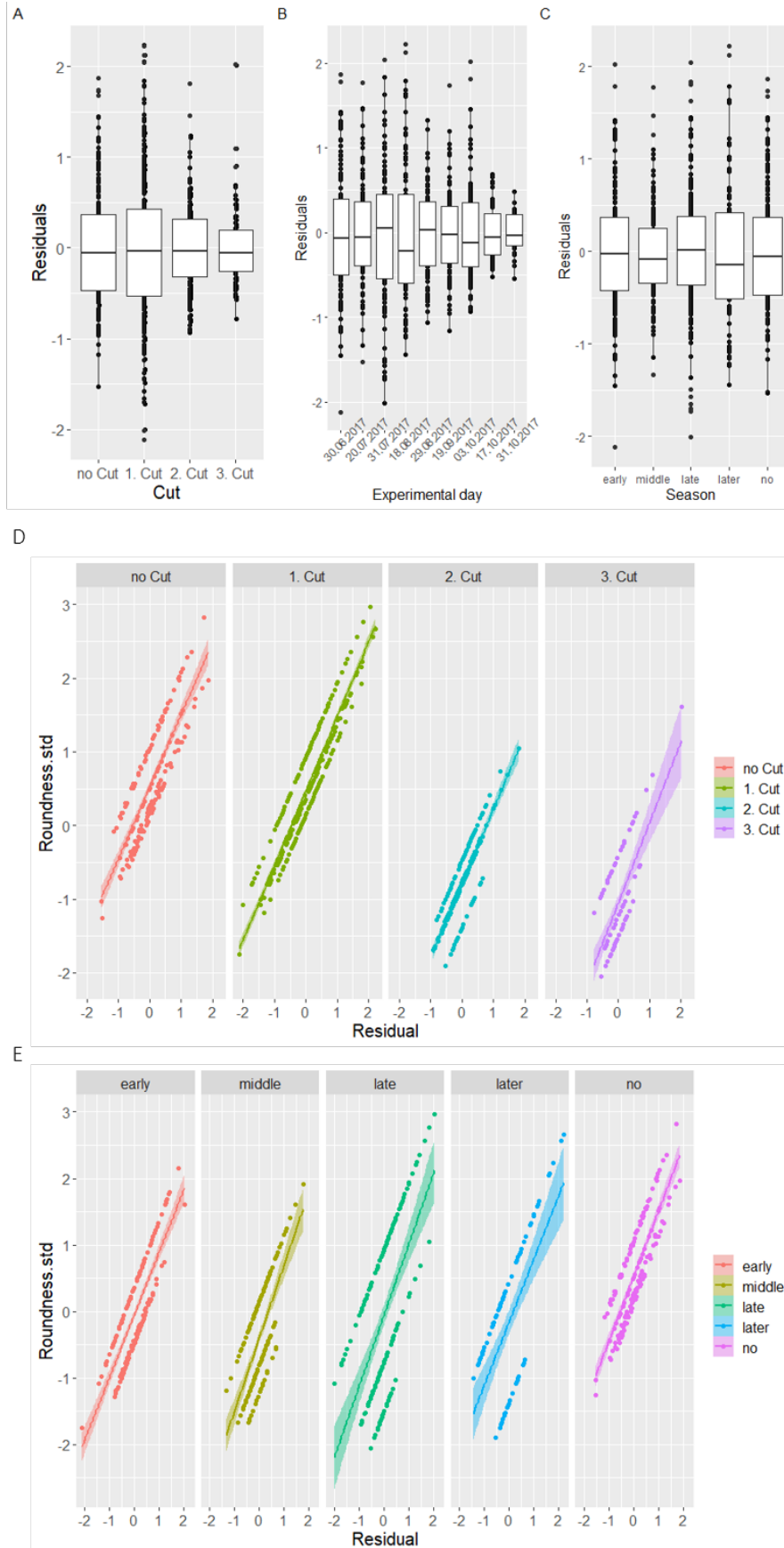
Supplementary material

Supplement Tab. 1: Model selection after ACI. Roundness of leaflets~ (selected model orange), Leaflet Area ~ (selected model green), df degrees of freedom, AIC Akaike information criterion, LogLik Log-Likelihood.

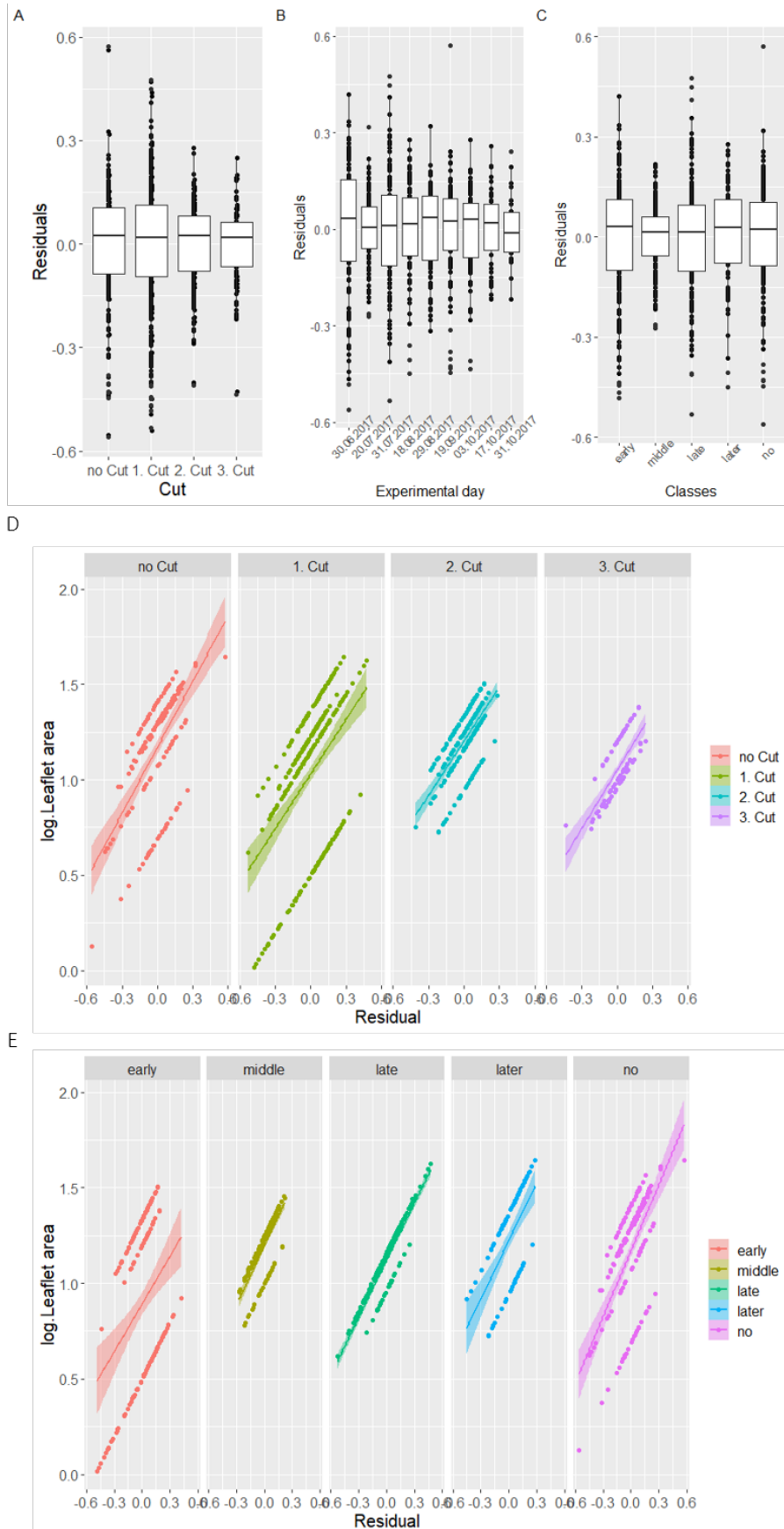
MODEL	Expression	LEAF LET ROUNDNESS			LEAF LET AREA		
		df	AIC	logLik	df	AIC	logLik
LM1	~ Date	10	317.13519	-148.6	10	5045.236	-2512.6
LM2	~ Classes	6	643.99998	-316	6	5642.2	-2512.6
LM3	~ Classes + Cut	9	NA	NA	9	5622.283	-2802.1
LM4	~ Date + Cut	13	67.67025	-20.8	13	5008.606	-2491.3
LM5	~ Date + Cut + (1 Classes)	14	65.11102	-18.6	14	5010.606	-2491.3
LM6	~ Date + (1 Cut)	11	90.67765	-34.3	11	5023.724	-2500.9
LM7	~ Date + (1 Cut) + (1 Classes)	12	80.65721	-28.3	12	5026.113	-2501.1
LM8	~ Classes + (1 Cut) + (1 Date)	8	97.74024	-40.9	8	5054.068	-2519
LM9	~ Date + (1 Patch)	11	102.66579	-40.3	11	5010.769	-2494.4
LM10	~ Classes + (1 Patch)	7	120.5131	-53.3	7	5066.344	-2526.2
LM11	~ Date + Classes + (1 Patch)	15	102.74644	-36.4	15	5010.808	-2490.4
LM12	~ Date + (1 Cut) + (1 Patch)	12	78.78658	-27.4	12	5011.891	-2493.9
LM13	~ Date + Classes + (1 Cut) + (1 Patch)	16	71.57755	-19.8	16	5012.808	-2490.4
LM14	~ Date + (1 Classes) + (1 Cut) + (1 Patch)	13	79.03038	-26.5	13	5013.891	-2493.9
LM15	~ Cut + (1 Date) + (1 Patch)	7	77.95899	-32	7	5032.99	-2509.5
LM16	~ Cut + Classes + (1 Date) + (1 Patch)	11	79.024	-28.5	11	5036.353	-2507.2



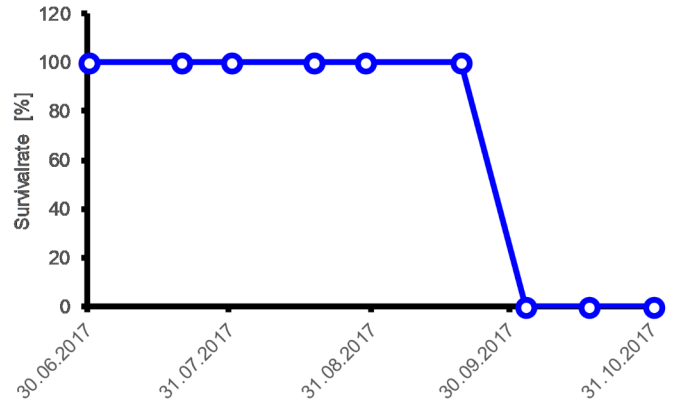
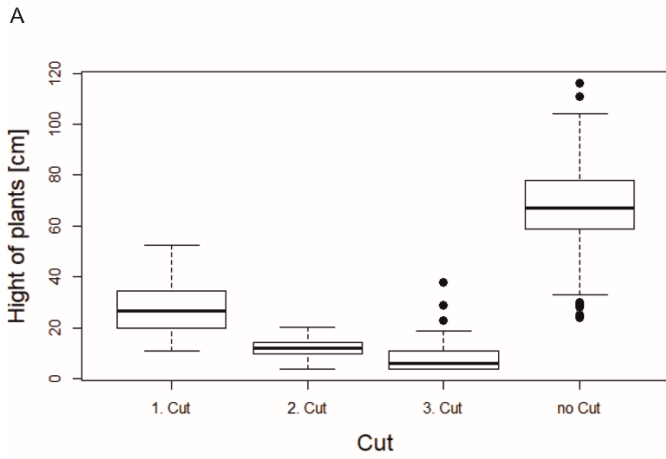
Supplement Fig. 1: Plotted residual and fitted values from model LM5 leaflet roundness upper row and leaflet area lower row. A: without transformation of leaflet roundness; B: after transformation with standardize of leaflet roundness; C: without transformation of leaflet area; D: transformed log leaflet area.



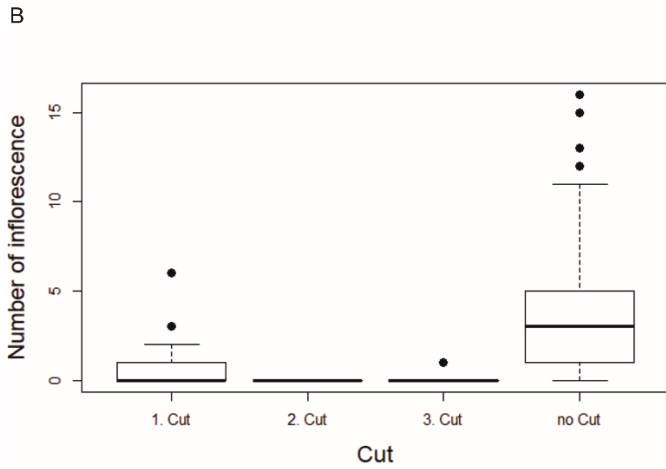
Supplement Fig. 2: Model LM5 validation after transformation of leaflet roundness. A: Residuals vs. Cutting events; B: Residuals vs. experimental Day (Days of moving events); C: Residuals vs. Classes (early, middle, late, later, and no~control); D: Roundness vs. Residuals in Cut events with smoothed regression line; E: Roundness vs. Residuals in Classes with smoothed regression line.



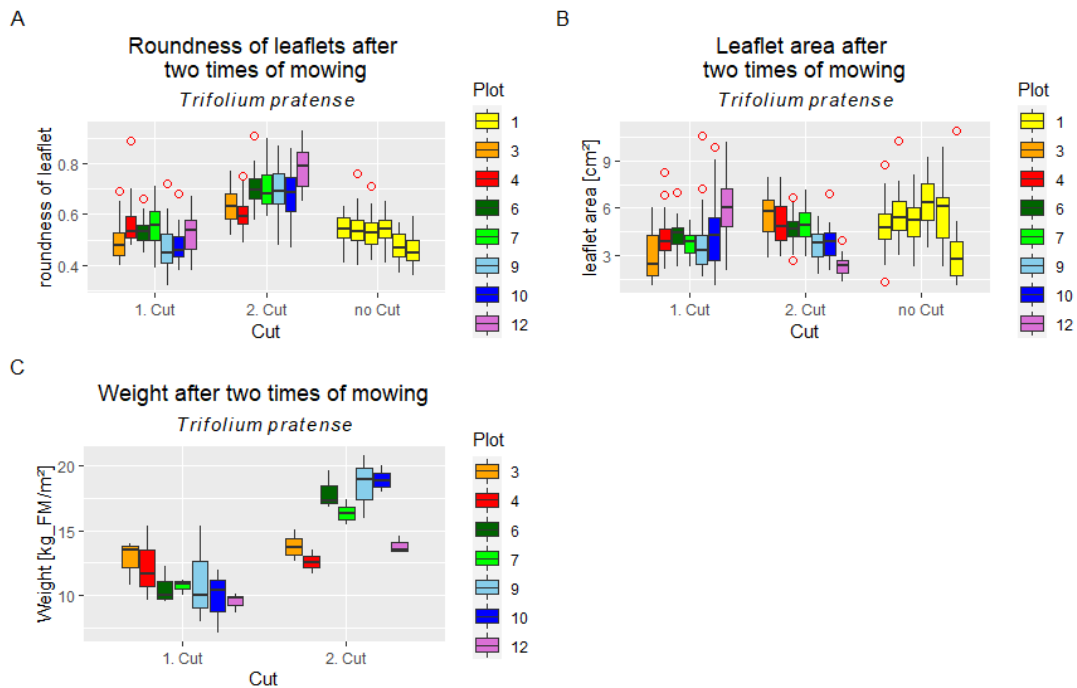
Supplement Fig. 3: Model LM4 validation after transformation of leaflet Area. A: Residuals vs. Cutting events; B: Residuals vs. experimental Day (Days of mowing events); C: Residuals vs. Classes (early, middle, late, later and no~control); D: Roundness vs. Residuals in Cut events with smoothed regression line; E: Roundness vs. Residuals in Classes with smoothed regression line.



Supplement Fig. 6: Survival rate of the control red clover plants during the field experiment.



Supplement Fig. 4: *Trifolium pratense* after the different mowing regimes. A: Height of plants; B: Number of inflorescences.



Supplement Fig. 5: *Trifolium pratense* after two times of mowing (regardless of the time) and the not mown control group. A: Roundness of leaflets in the different plots; B: leaflet area in the different plots; C: weight of the harvested biomass of the plots.