Sampling protocol of woody regeneration in the Pilis Experiments

Pilis Forestry Systems Experiment

(Updated: April 2022)

5 treatments:

C - control
CC - clearcutting
G – gap cutting
P – uniform preparation cutting
R – retention tree group

6 repetitions (blocks)
Altogether 30 plots

1) Survey of acorn production of sessile oak (*Quercus petraea*)

Counting the number of fallen acorns during November, in one 1 m × 1-m quadrate in each plot. Most of the fallen acrons are sessile oak, the number of Turkey oak (*Quercus cerris*) acorns are minimal, thus we does not separate them.
Comment (2018): Counting was done from 2014 until 2018 (but missed in 2017). 2018 was a masting year. Due to the huge number of acorns, in this year only 0.5 m × 0.5-m quadrates were used, and their data were multipled to 1 m^2.

2) Survey of the natural regeneration

In the understory quadrates, we also sample the woody regeneration. Two 2 m × 2-m permanent quadrates were designated in each plot, one in a fenced area and one outside the fence. Comparing the data of the two quadrates, the effect of game browsing can be evaluated. In the quadrates, we estimate the cover of woody species lower than 50 cm in percentage, similarly to the sampling of herbs). Besides, we count the number of sapling per species, in four size categories:

1. 0–20-cm height
2. 2–50 cm
3. 50–130 cm
4. >130 cm, DBH< 5 cm

This study is carried out since 2014 yearly, in summer.

3) Investigation of the spatial pattern of woody regeneration

This study is related to the survey of the spatial pattern of the understory, and is done in four blocks (1, 2, 3, 6). One circular sampling area was designated in each plot, with a diameter of 20 m. Within this area, 81 sampling points are arranged in a 2 m × 2-m grid, covering the whole area (Fig. 1). In the sampling points, we estimate the cover of woody species (smaller than 50 cm) in 0.5 m × 0.5-m quadrates. We also count the number of saplings for each woody species, in four size categories, similarly to the 2 m × 2-m quadrates. This survey is done in every second year (until date in 2016, 2018, and 2020).

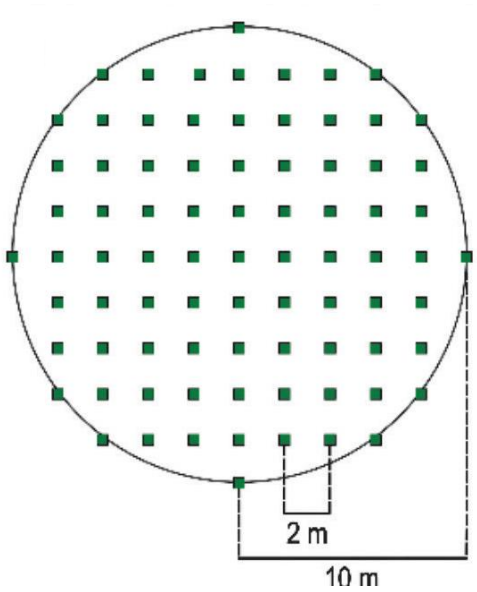

Figure 1. The sampling design for the study of the spatial pattern of the understory and woody regeneration, with 81 0.5 m × 0.5-m quadrates, in the Pilis Forestry Systems Experiment.

4) Survey of planted tree seedlings

Investigated tree species:

beech *(Fagus sylvatica*)
Turkey oak (*Quercus cerris*)
hornbeam (*Carpinus betulus*)
sessile oak (*Quercus petraea*)
common ash (*Fraxinus excelsior*)

We planted five seedlings of each species to each plot, to the fenced area. Planting was done in 2014 March, after the end of the ground frost. Seedlings were arranged in the 30 plots in a random way, the distance of seedlings from each other within the plots was usually 70 cm. All seedlings were marked with an individual identification code. The seedilgs died due to the planting stress were repleced by new ones in the spring of 2015.

Comment (2021): For the 7th year after the interventions, the shade-tolerant tree species (ash, hornbeam, and beech) grew substantially over the oak individuals, and due to their close position and shading effect their hampered the development of oaks. Since our study focuses basically to oaks, we closed the study of shade-tolerant species species (ash, hornbeam, and beech) with the dataset of the 2020 year, and logged these individual in the spring of 2021. Thus, the remained oak saplings can be further studied without the competition effect of shadig species.

Measured variables:

Length of the shoots: Measurement of the longest shoot (apical shoot, or a side-shoot that took its role) from the soil until the base of the apical bud. It is measured since 2014, in every year.

Diameter at ground level: Measurement of the diameter of the stem directly above the ground, with digital calliper. It is measured since 2014, in every year.

Number of shoots: Count of all shoots on the seedling that have at least one living leaf.

Comment (2018): With the growth of the seedlings it increased so much that on the larger individuals we counted the shoots only one larger branch, and multiplied it to the whole plant.

Comment (2019): We closed this measurement with the 2018 dataset, because on the larger individuals we could not count the huge number of shoots from 2019. Meanwhile it did not add too much extra information compared to the height measurement.

Leaf area: Measurement of the area of one selected average-sized leaf on each seedling, by a portable laser leaf area scanner (CID-202, CID Bio-Science, USA). The equipment was calibrated before the use as prescribed by the producer. With the multiplication of the measured leaf area with an estimated leaf number we received an estimation for the whole leaf area of the individual.

Comment (2019): We closed this measurement with the 2018 dataset, because on the larger individuals we could not count the huge number of leaves from 2019. Meanwhile it did not add too much extra information compared to the height measurement.

5) Investigation of the growth of individual seedling in the natural regeneration

In order to study the effect of game browsing, 188 pairs of seedlings are measured yearly among the individuals of the natural regeneration. These seedling are belonging to 12 woody species:

wild service tree (*Sorbus torminalis*)
beech (*Fagus sylvatica*)
Turkey oak (*Quercus cerris*)
common hawthorn (*Crataegus monogyna*)
hornbeam (*Carpinus betulus*)
sessile oak (*Quercus petraea*)
field maple (*Acer campestre*)
wild cherry (*Prunus avium*)
wild pear (*Pyrus pyraster*)

wild rose (*Rosa canina agg*.)
dogwood (*Cornus sanguinea*)
manna ash (*Fraxinus ornus*)

Among the pairs, one is located in the fenced area, and the other is outside the fence. At the start, the member of the pairs were in similar developmental stage. The number of pairs within a species was determined by the number of occurring individuals in the fenced area. We assigned the unfenced pairs to these designated fenced individuals.
On the seedlings, we measure size-variables and the evidence of browsing. In the current protocol, we describe only those variables that are analysed also independently from the browsing. The analysis of browsing is done in a separate project by colleagues. Measurements are done since 2014, in every summer.

Measured variables:
Length of the shoots: Measurement of the longest shoot (apical shoot, or a side-shoot that took its role) from the soil until the base of the apical bud.
Height: Height of the apical bud from the ground.
Diameter at ground level: Measurement of the diameter of the stem directly above the ground, with digital calliper.
Number of shoots: Count of all shoots on the seedling that have at least one living leaf.
Number of leaves: Count of all leaves on the seedling. In the case of large saplings, leaves are counted only one branch, and multiplied with an estimated multiplier.
Estimated leaf area: The length and width of one average leaf is measured, and its area is calculated, considering the shape of the leaf as an ellipse. With its multiplication with the number of leave we estimate the whole leaf area of the plant.

Pilis Gap Experiment

6 treatments:

CO – control
LC – large circular gap
LE –large elongated gap
SC –small circular gap
SE –small elongated gap
EX –extended gap: small circular gap, that will be extended after some years (when the regeneration of oak will be strong) to a large circular gap

6 replicates (blocks)
Altogether 36 plots
The whole area is fenced, thus regeneration can be studied without the effect of game browsing.

1) Survey of acorn production of sessile oak (*Quercus petraea*)

Counting the number of fallen acorns during November, in one 1 m × 1-m quadrate in each plot, in the center of the gap. Most of the fallen acrons are sessile oak, the number of Turkey oak (*Quercus cerris*) acorns are minimal, thus we does not separate them.
Comment (2020): 2020 was a slightly masting year. In this year, assigned to the spatially extended sampling, acorn was counted in the 41 0.5 m × 0.5-m quadrates, and their data were multipled to 1 m^2.
Comment (2021): In this year, the acorn sampling in the center was complemented with a second 1 m × 1-m quadrate at the northern border of the gaps. In the future, we plan to repeat the counting in years with various amount of acorns, in both 1 m × 1-m quadrates.

2) Survey of the natural regeneration

In the understory quadrates, we also sample the woody regeneration. One 2 m × 2-m permanent quadrate was designated in the center of each plot. In these quadrates, we estimate the cover of woody species lower than 50 cm (in percentage, similarly to the sampling of herbs). Besides, we count the number of sapling per species, in four size categories:

1. 0–20-cm height
2. 2–50 cm
3. 50–130 cm
4. >130 cm, DBH< 5 cm

This study is carried out since 2018 yearly, in summer.

3) Investigation of the spatial pattern of the woody regeneration

This study is related to the survey of the spatial pattern of the understory, and is done in four blocks (1, 3, 4, 5). We have 8 transects forming an asterisk, in the direction of the four main and four secondary directions (Fig. 2). Along the transects, altogether 41 0.5 m × 0.5 m quadrates are designated. We estimate the cover of woody species (smaller than 50 cm), and also count the number of saplings for each woody species, in four size categories, similarly to the 2 m × 2-m quadrates. This survey is done in every second year (until date in 2019 and 2021).

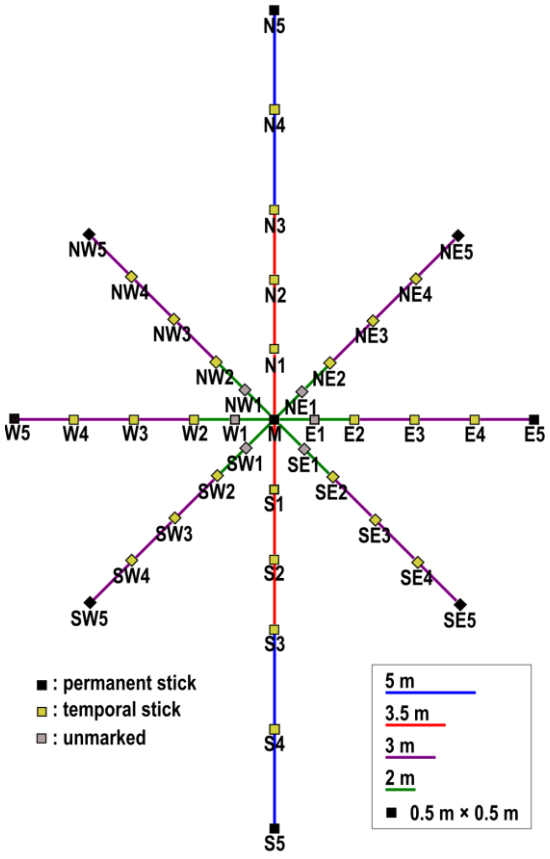

Figure 2. Transects for the investigation of the spatial pattern of the regenration (and understory herbs) in the Pilis Gap Experiment.

4) Measurement of the mortality of sessile oak seedlings

We count the number of sessile oak seedlings originated from the 2018 masting year (i.e. belonging to the same cohort). Counting is done in four 0.5 m × 0.5-m quadrates at the northern part of the gaps (which receives the highest direct light). The sum of the count in the four quadrates is assigned to the plot. Fresh seedlings emerged from acorns of the next years are removed during the calculation.

This study is carried out since 2019 yearly, in August-September.

5) Survey of the growth of individual sessile oak seedlings

Our aim was the standardized measurement of oak seedlings with the same age. Since 2018 was a masting year, we have a lot of natural, even-aged seedlings, thus it was not necessary to plant them artificially. In order to avoid the intensive initial mortality of the marked seedlings, individuals were assigned just one year after their emergence (in 2020). We marked 30 seedlings with individual codes in each plot, at the northern part of the gap, in a 3 m × 3-m area fenced for this study. 180 seedlings per treatment, i.e. altogether 1080 seedlings were marked. Thus, we expect a proper number of individuals for statistical analysis even after a mortality of the young trees. During the selection of the individuals, the main aspect was to be originated from the 2018 cohort (being even-aged), and to keep their initial size as similar to each other as possible (preferably 10-20 cm height). In certain plots, if we did not find enough seedlings from this size category, we also marked smaller or slightly larges seedlings. If it was possible, we kept a distance of 40 cm between the individuals. The blackberry *(Rubus fruticosus agg.*), and larger seedlings shading our individuals are regularly removed around our seedlings.

the distance of seedlings from each other within the plots was usually 70 cm. All seedlings were marked with an individual identification code. The seedilgs died due to the planting stress were repleced by new ones in the spring of 2015.
Comment (2021): For the 7th year after the interventions, the shade-tolerant tree species (ash, hornbeam, and beech) grew substantially over the oak individuals, and due to their close position and shading effect their hampered the development of oaks. Since our study focuses basically to oaks, we closed the study of shade-tolerant species species (ash, hornbeam, and beech) with the dataset of the 2020 year, and logged these individual in the spring of 2021. Thus, the remained oak saplings can be further studied without the competition effect of shadig species.

Measured variable:
Length of the shoot: Measurement of the longest shoot (apical shoot, or a side-shoot that took its role) from the soil until the base of the apical bud. It is measured since 2020, in every year, during August-September.
Comment (2021): in the autumn of 2021, we marked 82 new seedlings in place of the died ones.