LIFE Project Number

LIFE17 NAT/FI/000544

Final report March 2025

Consequences of restoration of semi-natural grasslands by grazing for tick abundance and tick-borne pathogens: a before-after-control-impact study

Actions: D2

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Summary

Semi-natural grasslands are the most threatened habitat in Finland and are threatened throughout the E.U. In general, conservation actions for maintaining grassland habitat – mainly mowing and grazing – are the main conservations actions done in the E.U. In CoastNet LIFE, semi-natural grasslands in islands in SW Finland and along the west coast of Finland were restored and grazers were introduced. Here we study the effect of this conservation action on ticks and diseases the ticks carry using a before-after-control-impact BACI design. We collected tick using cloth-dragging to estimate their abundance and using DNA/RNA methodology to infer disease prevalence of ticks on four pairs of islands (total eight islands) of the same habitat type both before (2019) and after (2024) semi-natural grassland on half of the islands started to be grazed (grazing started in 2022). We screened the ticks for *Borrelia* (several species), *Neoehlichia mikurensis*, *Rickettsia* spp, *Anaplasma phagocytophilum*, *Babesia* spp. and Tick Borne Encephalitis virus. Contrary to our expectation, we found no change in tick abundance or disease prevalence. However, as grazing started only in 2022 our study only considers effects of 2 years which is short-term given the tick life cycle is 3 years. We discuss implications and possible factors behind our findings.

Introduction

Semi-natural grasslands are the most threatened habitat type in Finland (Kontula & Raunio 2019). In the E.U., as a whole, grasslands are among the most threatened habitat types (EEA 2020). Semi-natural grasslands are habitats which were created and which require maintenance by humans. These habitats originated under the extensive agriculture practices traditionally practised during the last centuries. From an ecological and evolutionary perspective, this man-made habitat acts for many animal and plant species as a surrogate for the once natural grasslands maintained by now-extinct large grazers. Under extensive agricultural practices, sheep and cattle are primarily fed by grazing and hay made locally, thus sustaining a landscape with a sizeable fraction of grasslands. Agricultural intensification, however, drastically reduces that need leading to either the abandonment of traditional grasslands, where succession shifts the habitat to something else (forest in Finland), or its inclusion into crop production changes grassland into field.

Within Natura 2000 protected areas, most conservation actions carried out in the E.U. revolve around maintaining some form of extensive agricultural practices where, for semi-natural grasslands, mowing and grazing are primary tools to safeguard this habitat from succession (EEA 2020). In addition, semi-natural grasslands are a potential target for restoration actions. This is because succession is a slow ecological process and after abandonment, at least the physical characteristics of semi-natural grasslands are maintained for decades (although actual species that are highly dependent on these semi-natural grasslands may not persist). Restoration is, because of the E.U. habitat restoration act, anticipated to become more common. By its very nature of causing a change in the landscape, habitat restoration has a socio-economic dimension as it affects stakeholders such as local residents, local enterprises and visitors. Effects of restoration may be positive, an increase in the perceived nature value. However, restoration may also have negative effects.

As part of the CoastNet LIFE project, restoration of semi-natural grasslands were conducted in the Archipelago National Park and along the Finnish south-west coast. Restoration of these semi-natural grasslands includes opening up the habitat by removal of some large trees and shrubs, fencing the area and introducing medium- or large-sized mammalian grazers (e.g. sheep, cattle). These grazers are maintenance hosts for hard ticks (Acari: Ixodidae), and are competent hosts for the zoonotic pathogens ticks carry, including *Borrelia* spirochetes. In Finland, the abundance of ticks has dramatically increased in recent decades, especially in the archipelago of southern Finland (Sormunen et al. 2016a, 2020a). Also there is an increase in the incidence of borreliosis as well as tick-borne encephalitis (TBE), which are diseases caused by bacteria and a virus, respectively, transmitted by ticks from host animals to humans (Sajanti et al. 2017, Smura et al. 2019). Due to these strong changes in tick abundance and disease numbers, the general public in Finland is nowadays well aware of ticks and diseases they cause (Gould et al. 2025). Public opinion is also vested against animals, which putatively increase abundance of ticks. For example, the increase in tick abundance coincides with the increases in white-tailed deer (*Odocoileus virginianus*) and roe deer (*Capreolus capreolus*) abundances during the last decades, and public concerns have been raised over tighter control of deer populations as a way to reduce ticks. From this perspective, conservation actions of semi-natural grasslands, including the introduction of grazers, on public land may cause concern of the public about potential negative effects these actions may have on ticks and pathogens ticks transmit.

The life-cycle of hard ticks is complex and lasts several years. In principle, ticks have a three-year life-cycle (exceptions are possible), feeding each year once on a host. In their first year of life, tick larvae are small and they primarily feed on small mammals or passerines, although they can – when abundance is high – feed also on larger animals. In their second and third year, when ticks are nymphs and adults, respectively, they take their bloodmeals also from larger animals. As a rule, ticks become carriers of *Borrelia* (and most other pathogens) only if they feed on a host that is infected. Thus, tick larvae are not carriers of *Borrelia*, but become carriers after they have fed on a pathogen-carrying host (e.g., a vole) and can then infect other hosts (including humans) in the next year as a nymph. Because adults are relatively rare, most studies of tick-borne pathogens (including this one) focus on tick nymphs and whether they carry a pathogen or not. Furthermore, as they are relatively small-sized (1.5 mm), nymphs are difficult to notice and remove from skin, emphasising their crucial role as pathogen vectors to humans and pet and domestic animals. Only in the adult stage do ticks reproduce (a female may lay up to 2000 eggs), but any *Borrelia* they carry as adults is typically not transmitted to their offspring.

From an ecological perspective, the relationship between abundance of grazers and abundance of ticks and the pathogens they carry is not immediately obvious. This is because the consequences of restoration and grazing of semi-natural grasslands depends on both direct and indirect effects these actions would have. The direct effect is that the addition of grazers provides more opportunity for ticks to obtain a bloodmeal (i.e. there is more food for ticks) which, assuming this has been a limiting factor on tick population growth, is expected to increase the abundance of ticks. The indirect effect of habitat restoration and grazing actions is that – by definition – this action changes the habitat which may affect the hosts of ticks. For example, reduction of grass by grazing reduces the food resource available for grass-eating voles and may increase predation pressure (less cover), thereby reducing the abundance of voles which are the primary food resource for larval ticks. A reduction in the abundance of voles may lead to a dilution effect in *Borrelia* transmission to the larval ticks as these will proportionally feed more on other species of hosts that do not carry *Borrelia*, assuming their abundance is impacted less than voles are. Ecologically, therefore, the final outcome of restoration of semi-natural grasslands and introduction of grazers will depend on how strong these direct (presumably positive) effects and indirect (presumably negative) effects are on ticks and the pathogens they carry. Grazing by large animals and habitat restoration may also change microclimate experienced by ticks in the vegetation and litter layer. Generally ticks thrive better under shadows and in relatively moist conditions, while direct sunshine and heat have a negative effect on the activity of ticks. Thus, already opening up the habitat by removal of shrubs and trees as part of restoration effort of semi-natural grasslands could impact ticks negatively.

Given the uncertainty in how restoration and grazing of semi-natural grasslands could affect tick abundance and pathogens tick transmit, there is public and economic interest to better understand what consequences restoration and introduction of grazers of semi-natural grasslands could have in this respect. As part of CoastNet LIFE project, we aimed to study these effects using a before-after control-impact design.

Material and Methods

Study islands and collection of ticks

Tick sampling was conducted on eight islands/peninsula (Ejskäret, Hevonkack, Kenkämaa, Långholmen, Omenapuumaa 1, Omenapuumaa 2, Pyytti and Sundholm) located in the Baltic Sea both in June 2019 and again in June 2024 (**Table 1**). In actual sampling transects chosen to represent natural tick habitat on each island, questing ticks were collected using a standard cloth-dragging method, in which a 1-m^2 white cotton cloth was dragged ten metres through ground vegetation at a slow walking pace (Nyrhilä et al. 2020; **Figure 1**).

Figure 1. Tick collection using the cloth-dragging method in a control area (not restored semi-natural grassland).

Ticks that attached to the cloth were counted according to their developmental stage and sex (larva, nymph, adult male or adult female) and preserved in ethanol-filled Eppendorf tubes and stored at -20°C for further analyses. Fifteen to 40 transect repetitions were conducted on each island per sampling year, but actual 10-m transects were not marked and thus they were not exactly the same in 2019 than in 2025 within the sites. According to our knowledge, *Ixodes ricinus* (common name: the sheep tick or castor bean tick) is the only exophilic *Ixodes* species occurring in south-western Finland, including our study islands and the archipelago nearby (Sormunen et al. 2016a, b, c, 2018, Klemola et al. 2019, Laaksonen et al. 2017, 2018).

Table 1. Study islands, tick sampling schedule and mean (± standard deviation) numbers of ticks (*Ixodes ricinus*) sampled per 10-m cloth-drag transect in 2019 and 2024. Column N gives the number of drags per island. Grazing manipulation was ongoing on four islands in 2022 and 2023. Coordinates (by WGS 84) denote locations of the islands in the Baltic Sea but do not point to exact sampling transects of ticks within islands. In brackets for each island the national habitat letter code (type of semi-natural grassland) is provided with a numerical code to indicate the pairing of islands deemed to be similar.

Island	Year	Manipulation	Dev. stage	N	Mean	Std. Dev.
Ejskäret N: 59.888° E: 22.577° (Hk 12)	2019 20 Jun	No	Adults	15	0.47	0.92
		No	Larvae	15	57.20	68.98
		No	Nymphs	15	8.87	6.59
	2024 7 Jun	Grazing in 22/23	Adults	15	1.13	0.99
		Grazing in 22/23	Larvae	15	27.27	35.83
		Grazing in 22/23	Nymphs	15	9.87	6.53
Hevonkack N: 60.335° E: 21.590°	2019 18 Jun	No	Adults	25	0.32	0.69
		No	Larvae	25	9.60	11.59
		No	Nymphs	25	2.44	1.98
		No	Adults	25	0.16	0.47

(Hk 11)	2024 27 Jun	No	Larvae	25	16.72	22.95
		No	Nymphs	25	1.88	2.17
Kenkämaa N: 60.335° E: 21.687° (TrNi 8)	2019 17 Jun	No	Adults	40	0.53	0.99
		No	Larvae	40	20.78	16.63
		No	Nymphs	40	3.73	5.92
	2024 24 Jun	Grazing in 22/23	Adults	40	0.13	0.40
		Grazing in 22/23	Larvae	40	7.90	11.01
		Grazing in 22/23	Nymphs	40	1.50	1.99
Långholmen N: 59.884° E: 22.543° (Hk 12)	2019 20 Jun	No	Adults	15	0.47	0.64
		No	Larvae	15	84.53	85.72
		No	Nymphs	15	10.73	7.86
	2024 7 Jun	No	Adults	15	4.40	3.09
		No	Larvae	15	121.07	89.27
		No	Nymphs	15	31.00	18.37
Omenapuumaa 1 N: 61.192° E: 21.460° (Hk 10)	2019 27 Jun	No	Adults	20	0.05	0.22
		No	Larvae	20	0	0
		No	Nymphs	20	0.15	0.37
	2024	Grazing in 22/23	Adults	20	0.10	0.31
		Grazing in 22/23	Larvae	20	0.25	0.79

	10 Jun	Grazing in 22/23	Nymphs	20	0.45	0.60
Omenapuumaa 2 N: 61.188° E: 21.458° (Hk 10)	2019 27 Jun	No	Adults	15	0	0
		No	Larvae	15	0	0
		No	Nymphs	15	0.07	0.26
	2024 10 Jun	No	Adults	15	0.13	0.35
		No	Larvae	15	0.33	1.29
		No	Nymphs	15	2.93	2.02
Pyytti N: 60.366° E: 21.665° (TrNi 8)	2019 19 Jun	No	Adults	20	0	0
		No	Larvae	20	8.35	18.88
		No	Nymphs	20	1.70	2.00
	2024 25 Jun	No	Adults	20	0.10	0.31
		No	Larvae	20	2.75	8.12
		No	Nymphs	20	1.85	2.01
Sundholm N: 60.282° E: 21.320° (Hk 11)	2019 1 Jul	No	Adults	15	0.27	0.46
		No	Larvae	15	3.73	10.44
		No	Nymphs	15	1.27	1.62
	2024 26 Jun	Grazing in 22/23	Adults	15	0	0
		Grazing in 22/23	Larvae	15	0.20	0.41
		Grazing in 22/23	Nymphs	15	0.53	1.19

Grazing manipulation

Cattle or sheep were introduced to Ejskäret, Kenkämaa, Omenapuumaa 1 and Sundholm first time in summer 2022 and again in summer 2023. Species and numbers of animals and the times of grazing markedly varied among islands (**Table 2**), and thereby also varied the realized impact of the grazing manipulation on the vegetation and landscape. Cattle and sheep were medicated against ticks (**Table 2**).

Table 2. Cattle and sheep were introduced as grazers on study islands in 2022 and 2023.

Island	Grazers (approx. abundance, no. of ind.)	Time of grazing	Medication of grazers against ticks*
Ejskäret	2022: sheep (11- 25 [adults & lambs]) 2023: sheep (7 - 33 adults)	2022: mid Jun - mid Sep 2023: mid May - mid Oct	2022: Spotinor 2023: n.a.
Kenkämaa	2022: cattle (9 adults) 2023: cattle (26 [adults & calves])	2022: early Jul - mid Sep 2023: late Jul - mid Sep	2022: Coopersect 2023: Coopersect & Spotinor
Omenapuumaa 1	n.a.	n.a..	n.a.
Sundholm	2022: cattle (6 adults) 2023: cattle (9 adults)	2022: late Aug - mid Oct 2023: mid Aug - late Sep	2022: none 2023: Coopersect & Spotinor

* Spotinor and Coopersect are deltamethrin spot on solutions for the treatment and prevention of infestations by ticks, lice, flies, etc. on cattle and sheep.

DNA based identification of pathogens

We screened the sampled nymphs and adult ticks for the presence of TBEV and most significant bacterial and protozoan pathogens of human or veterinary importance. We ignored larvae as these have carried very few pathogens in earlier studies globally. As said in the Introduction, this is because questing larvae have not taken their first blood meal yet, and the transmission from parent to offspring via the ovaries occurs rarely or 'never' depending on the pathogen. The screened bacterial pathogens included the Lyme borreliosis agent *Borrelia burgdorferi* sensu lato group (*B. afzelii, B. burgdorferi* sensu stricto*, B. garinii*, *B. valaisiana* (only in 2019) and some unconfirmed ones), a tick-borne relapsing fever spirochete *Borrelia miyamotoi*, an agent for human granulocytic anaplasmosis *Anaplasma phagocytophilum*, spotted fever agents *Rickettsia spp.*, *Neoehrlichia mikurensis* (neoehrlichiosis), *Bartonella spp.* (e.g., cat scratch disease) and *Francisella tularensis* (tularemia). Protozoan parasites *Babesia spp.* (babesiosis) were also screened.

Total DNA and RNA was extracted from ticks using NucleoSpin96 RNA Kit with NucleoSpin® RNA/DNA Buffer Set (Prod id 740709 and 740944 Macherey-Nagel, Germany) according to the kit protocols (02/2023, Rev. 10). Extracted DNA and RNA was stored at −20°C and −80°C, respectively, until analysis.

For *Borrelia*, all DNA samples were first analyzed for *B. burgdorferi* s.l. (using primers Bb23Sf+r), and individual samples from a pool found positive were subsequently re-analyzed separately for genospecies *B. afzelii, B. burgdorferi* s.s.*, B. garinii, B. valaisiana* (in 2019) and *B. miyamotoi.* Pathogens with low expected prevalence *(Bartonella* spp.*, F. tularensis, Rickettsia* spp.*, N. mikurensis, Babesia* spp. and *A. phagocytophilum*), were first analyzed in pools (8 samples per pool, 5 µl of each sample). Individual samples from a pool found positive were subsequently re-analyzed separately. Real-time quantitative PCR (qPCR) reactions were performed using SensiFAST™ Probe Lo-ROX (for DNA) and SensiFAST™ Probe Lo-ROX One-Step Kits (for RNA) (Meridian Bioscience, Germany), and amplified on a QuantStudio 12K Flex Real-Time PCR System. All DNA samples were analysed using two replicate reactions performed in 96- or 384-well plates. At least one blank water sample (with replicate) was used as negative control in each assay. Commercial Amplirun DNA controls of *B. afzelii*, *Bartonella henselae, F. tularensis* and *Rickettsia conorii* (Viricell, Spain), were used as positive controls for the respective pathogen genera. For *B. miyamotoi, B. garinii* and *B. burgdorferi* s.s., samples previously identified as positive via qPCR analysis were used. The samples were considered positive only when a successful amplification was detected in both replicate reactions. Protocols for real-time quantitative PCR (qPCR) assay protocols for detection of the pathogens, followed our previous works (Sormunen et al. 2016a, 2018, 2020a, b; Laaksonen et al. 2017, 2018; Klemola et al. 2019). For detailed information on primer/probe sequences and amplification protocols see Online Resource 1 in Nyrhilä et al. (2020). All laboratory work for DNA/RNA-extraction and preparation of the qPCR reactions were done at the Center of Evolutionary Applications (University of Turku). qPCR amplification was performed at FFGC, Turku Bioscience Center.

Statistical analyses

Because the occurrence and abundance of questing tick larvae are clustered in time and space according to egg laying behaviour of adult females, and thereby have high and unpredictable variation within and between the sampling sites (see **Table 1**), we conducted statistical tests on tick abundance only for nymphs and adults. Furthermore, patterns in the pathogen prevalence were statistically analyzed for nymphs only, and these tests considered only the most prevalent pathogen species in which enough large numbers of positive detections were observed. In other words, the relatively low number of screened adults and/or that of positive detections prevented us from conducting meaningful statistical tests for the adults and for the pathogen species/genera that were (too) rarely detected.

Tick abundance (response was tick count per single 10-m cloth-dragging transect; separately for adults and nymphs) was modelled using a generalized linear mixed model (GLMM) with a negative binomial error distribution and log link function. Year, manipulation and their interaction were set as fixed factors, while island (nested within a year) with multiple transects was set as a random intercept effect. The probability that a nymph will be positive for *B. burgdorferi* s.l. (or for *B. afzelii*, *Rickettsia* spp. or *A. phagocytophilum* in separate models) was modelled using a GLMM with a binary error distribution and logit link function. For the pathogens, the same fixed and random effects [i.e., island nested within a year to control for multiple samples from the same location] were used than for the tick abundance. It should be noted that examination of the interaction between a time point (2019 or 2024) and manipulation (grazing or control) is the 'point of interest' in the before-after-control-impact studies like ours. Model-derived, least-squares means (i.e., estimated marginal means that were back-transformed from the model (log/logit) scale to data scale) with their asymmetric 95% confidence intervals are given throughout the results. Kenward-Roger approximation was used for denominator degrees of freedom as recommended by Stroup (2013). All the GLMMs were run with the GLIMMIX procedure in SAS v. 9.4 (Stroup 2013).

Results

Tick Abundance

Mean abundances (per 10-m cloth-dragging) of ticks on the islands are given in **Table 1** above. Mean values < 1 can be considered as low or moderate, 1 – 10 as high and > 10 as extremely high tick abundance. As usual, larvae were most abundant, followed by nymphs and adults (**Table 1**).

Although the lowest nymph abundance was observed on four grazed islands in 2024 (**Figure 2A, Table 1**), the conducted GLMM indicated neither statistically significant interaction effect (Year × Manipulation: $F_{1,\,11.21}$ = 0.54, P = 0.476) nor main effects (Year: $F_{1,\,11.21}$ = 0.26, P = 0.622; Manipulation: $F_{1,\,11.21}$ = 0.41, P = 0.536). No statistically significant fixed effects were found for the adult abundance either (**Figure 2B**; Year × Manipulation: $F_{1,\,11.16}$ = 1.08, P = 0.321; Year: $F_{1,\,11.16}$ = 0.16, P = 0.696; Manipulation: $F_{1,\,11.16}$ = 0.01, P = 0.945). According to likelihood-ratio tests, however, the random effect (island nested within a year) explained among-island variation, independently of grazing manipulation, in abundances of both nymphal and adult ticks (χ^2_1 = 152.19, P < 0.0001 and χ^2_1 = 61.78, P < 0.0001, respectively).

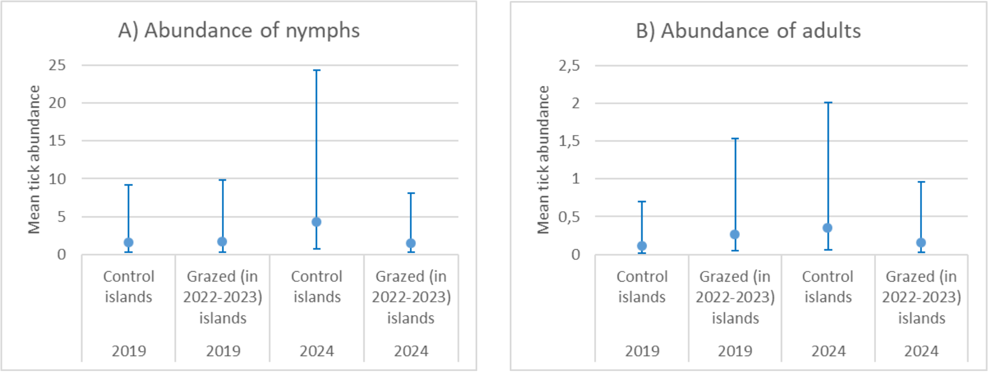

Figure 2. Mean estimated tick abundance (with 95% confidence interval) of nymphs (A) and adults (B) on islands before (in 2019) and after (in 2024) grazing manipulation.

Pathogen prevalence

Overall prevalence, separated for nymphal and adult stages but pooled for both study years, of screened pathogen species/genera are given in **Table 3.** No positive *Bartonella* spp. or *Francisella tularensis* detections were observed among nearly 1300 samples. Only one nymph was positive for TBEv (**Table 3**).

Table 3. Overall pathogen prevalences by development stage and sex of screened ticks.

Pathogen	Stage, sex	No. of positives	No. of screened	Prevalence percentage (95% binomial confidence interval)
***Borrelia burgdorferi* s.l.**	Nymph	86	1122	7.66 % (6.18 - 9.38)
↓	Adult, female	5	76	6.58 % (2.17 - 14.69)
↓	Adult, male	6	88	6.82 % (2.54 - 14.25)
Borrelia garinii	Nymph	14	1122	1.25 % (0.68 - 2.08)
↓	Adult, female	0	76	0 % (0 - 4.74)
↓	Adult male	2	88	2.27 % (0.28 - 7.97)

Borrelia valaisiana (2019 only)	Nymph	1	587	0.17 % (0 - 0.95)
↓	Adult, female	0	33	0 % (0 - 10.58)
↓	Adult, male	0	32	0 % (0 - 10.89)
***Borrelia burgdorferi* s.s.**	Nymph	17	1122	1.52 % (0.89 - 2.41)
↓	Adult, female	3	76	3.95 % (0.82 - 11.11)
↓	Adult, male	0	88	0 % (0 - 4.10)
Borrelia afzelii	Nymph	49	1122	4.37 % (3.25 - 5.73)
↓	Adult, female	1	76	1.32 % (0.03 - 7.11)
↓	Adult, male	3	88	3.41 % (0.71 - 9.64)
Borrelia miyamotoi	Nymph	4	1122	0.36 % (0.10 - 0.91)
↓	Adult, female	0	76	0 % (0 - 4.74)
↓	Adult, male	0	88	0 % (0 - 4.10)
Neoehrlichia mikurensis	Nymph	12	1122	1.07 % (0.55 - 1.86)
↓	Adult, female	1	76	1.32 % (0.03 - 7.11)
↓	Adult, male	1	88	1.14 % (0.03 - 6.17)
***Rickettsia* spp.**	Nymph	20	1122	1.78 % (1.09 - 2.74)
↓	Adult, female	4	76	5.26 % (1.45 - 12.93)
↓	Adult, male	2	88	2.27 (0.28 - 7.97)

Anaplasma phagocytophilum	Nymph	30	1122	2.67 % (1.81 - 3.80)
↓	Adult, female	6	76	7.89 % (2.95 - 16.40)
↓	Adult, male	3	88	3.41 % (0.71 - 9.64)
TBE virus	Nymph	1	1122	0,09 % (0 - 0.50)
↓	Adult, female	0	76	0 % (0 - 4.74)
↓	Adult, male	0	88	0 % (0 - 4.10)
***Babesia* spp.**	Nymph	2	1122	0.18 % (0.02 - 0.64)
↓	Adult, female	2	76	2.63 % (0.32 - 9.18)
↓	Adult, male	0	88	0 % (0 - 4.10)

Numbers of positive detections in nymph samples allowed statistical modelling by GLMMs for *B. burgdorferi* s.l., *B. afzelii*, *Rickettsia* spp. and *A. phagocytophilum* only, although broad 95% confidence intervals (partially due to low number of study islands) challenged also their interpretation (**Table 4, Figure 3**). It can be seen that *Borrelia* prevalence, and thereby the probability of a nymph to be positive for the *Borrelia* pathogens, were the lowest on four grazed islands in 2024 (**Figures 3A, 3B**). On the contrary, nymphs from the same grazed islands had the highest probability to be positive for *A. phagocytophilum* in 2024 (**Figure 3D**). However, no statistically significant Year × Manipulation interaction was detected for any of the analyzed pathogens (**Table 4**). The random effect [island (year)] was significant for the *B. burgdorferi* s.l. and *B. afzelii* (**Table 4**), indicating variation among islands independently of the grazing manipulation.

Table 4. Statistical values (F-statistic with numerator and denominator degrees of freedom and P-value) of conducted GLMMs for the probability of a nymph to be positive for different pathogens (four response variables in columns). Kenward-Roger approximation enables decimals for denominator DFs. Significance of the random effect (island nested within a year) to explain variation in the probability was tested by likelihood-ratio test.

Response → **Fixed factors ↓**	*B. burgdorferi* s.l.	*B. afzelii*	*Rickettsia* spp.	*A. phagocytophilum*
Year	$F_{1,\,9.579} = 2.48$ $P = 0.145$	$F_{1,\,15.61} = 3.37$ $P = 0.085$	$F_{1,\,4.295} = 0.47$ $P = 0.527$	$F_{1,\,4.508} = 2.18$ $P = 0.206$
Manipulation	$F_{1,\,9.579} = 5.59$ $P = 0.041$	$F_{1,\,15.61} = 3.59$ $P = 0.077$	$F_{1,\,4.295} = 0.09$ $P = 0.775$	$F_{1,\,4.508} = 1.69$ $P = 0.256$
Year × Manipulation	$F_{1,\,9.579} = 0.53$ $P = 0.486$	$F_{1,\,15.61} = 1.80$ $P = 0.199$	$F_{1,\,4.295} = 0.01$ $P = 0.908$	$F_{1,\,4.508} = 1.49$ $P = 0.283$
Random factor ↓				
Island (Year)	$\chi^2_1 = 6.33$ $P = 0.006$	$\chi^2_1 = 8.98$ $P = 0.001$	$\chi^2_1 = 0.70$ $P = 0.201$	$\chi^2_1 = 0.26$ $P = 0.305$

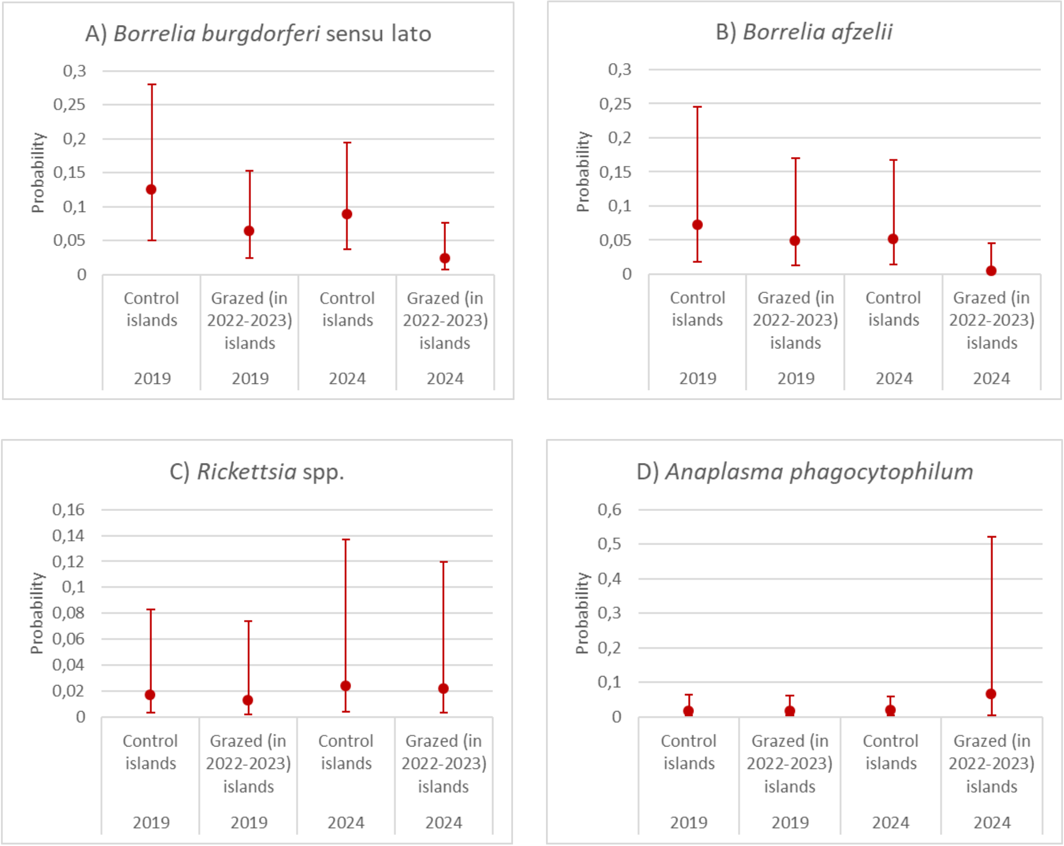

Figure 3. Mean estimated probability (with 95% confidence interval) of a nymph to be positive for *B. burgdorferi* s.l. (A), *B. afzelii* (B), *Rickettsia* spp. (C) and *A. phagocytophilum* (D) before (in 2019) and after (in 2024) grazing manipulation.

Conclusions and Recommendations

Tick abundance and *B. burgdorferi* s.l. prevalences were the lowest on four grazed islands in 2024. This indicated that the introduction of cattle and sheep on grazed islands in 2022/2023 did not seem to lead to higher tick abundance or increase the probability that ticks carried *Borrelia* bacteria or other pathogens. Although conservation actions of semi-natural grasslands, including the introduction of large grazers, on public land may understandably cause concern of the public about potential negative socio-economic effects due to increased amounts of ticks and the pathogens ticks transmit, our findings rather indicated the opposite: negative socio-economic effects were not found, instead, the tick abundance seemed to decrease on the grazed islands.

We do not have any straightforward explanation for the findings above, and naturally, they could also be observations just by chance due to the low amount of island replicates. It should also be noted that no statistically significant (interaction Year × Manipulation) effects were found for the tick abundance or pathogen prevalence. Anyway we could speculate, for example, that large grazers, cattle and sheep, disturbed smaller animals (e.g. voles, mice, squirrels, shrews, passerines (thrushes), hares, raccoon dogs, foxed, badgers, etc.), which are important and competent reservoir hosts for the pathogens, and thereby limited ticks as pathogen vectors. Large grazers could also disturb foraging and movements of medium- and large-sized maintenance hosts, such as deer, on the islands. Thus, the habitat restoration including the introduction of grazers may, through their indirect effect on other hosts, reduce the total amount of hosts available to ticks. A second, non-mutually exclusive explanation is that restoration of semi-natural grasslands have a direct effect on ticks. Possible direct pathways include the fact that prior to the introduction of grazers, bushes and trees are removed to provide space for the grazers and thus alter the habitat. Once grazers were introduced all vegetation consumption and trampling by cattle and sheep modified habitat further, leading to probable increase of heat and lessened moisture on the soil surface. As ticks do not thrive under (too) dry conditions, restoration of semi-natural grasslands (including trampling) may reduce the quality of the habitat for ticks. Lastly, grazers receive medication against ticks which acts to reduce their “capacity” as hosts for ticks. Hence, while introduction of grazers leads to more potential tick hosts, the net effect may, because of medication, be relatively small.

Unfortunately, we do not know what happened to host animal distribution and abundance, when large grazers were introduced. In future investigations, camera trappings (by wildlife cameras) are needed to obtain estimates of abundance of medium- and large-sized animals on the islands. Smaller mammals (especially voles and mice) should be live-trapped to get estimates on their numbers and activity. Shrews, as protected mammals, form a problem in this sense. Numbers of passerine birds can be estimated by line or spot censuses. All these field works are laborious and expensive to conduct with sufficient replication. However, using these methods one would obtain a better understanding of why ticks and tick-borne diseases may (at least in short term) decline after semi-natural grassland restoration and grazing.

Scientifically, better metadata on introduced grazers are needed. We should have clear data to show when, where, which species and how many grazers were used. In addition, a well-planned experimentation would be needed with designed and accurate set-ups. For instance, the medication of large grazers (if used) should be consistent among the study sites so that it does not cause unnecessary variation for the results. Realized grazing impact should also be well measurable on grazed islands during and after each summer.

The number of islands (4 grazed + 4 ungrazed) was rather low for a scientific study. To get statistically significant results, the number of islands should be at least two-fold, preferably three-fold, compared to this approach. Naturally this would demand massive amounts of field and lab work for the tick sampling, pathogen screening and monitoring of grazers and other animals by camera- and live-trappings. Also the transportation logistics in the archipelago could be a challenge. Ideally, ticks should be sampled 2-4 times per summer. The study should continue several years after the grazing manipulation, which itself should last 3-4 years, so that the whole three-year life-cycle of ticks is affected.

What comes to ticks and tick-borne pathogens, we conclude that any indication of harmful consequences was not found on the islands that experienced restoration of semi-natural grasslands by the introduction of large grazers. Scientifically this study can be seen as a pilot before more ambitious experimentation.

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