The effect of prescribed burning on *Pulsatilla vernalis* at Marma military training area in Sweden

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SUMMARY

Changes in habitat have led to a decline in many species which are now threatened. One of them is the spring pasque flower *Pulsatilla vernalis*, which grows on well-drained soils and is sensitive to competition. The species has in the past benefited from disturbances such as grazing, mowing and forest fires. Now that these do not occur as frequently, it has been suggested that prescribed burning could be used as a conservation intervention to benefit *P. vernalis.* In this study, we tested whether prescribed burning in 2018 benefited a population of *P. vernalis* at Marma military training area, outside Älvkarleby in eastern Sweden. Due to unexpected windy conditions on the day of the prescribed burning, not all the planned area was burned. This created a natural experiment that enabled us to compare burned areas with unburned areas (control) in both heathland and forested heathland habitats. The study includes data gathered before and after the experimental treatment. We found that compared to the control areas, the burned areas had a significantly higher number of *P. vernalis* tufts (clusters of leaf rosettes), as well as a greater number of flower stalks per tuft. Although limited due to lack of replication, this study supports the suggestion that prescribed burning benefits *P. vernalis*, both in open areas as well as in forests.

BACKGROUND

Human activity on our planet has led to a decline in biodiversity (Ceballos *et al.* 2015, Diáz *et al.* 2019, Ceballos *et al*. 2020). Globally, the largest cause of extinction is human alteration or destruction of habitats (Dirzo & Raven 2003). Modern forestry and modern agriculture have replaced traditional land management in many parts of the world, which has disadvantaged several species (Poschlod *et al.* 2005). In addition, abandonment of less productive land often leads to loss of open habitat (Levers *et al*. 2018). One of the plant species that is negatively affected by that is the spring pasque flower *Pulsatilla vernalis* (Stridh *et al.* 2016).

Pulsatilla vernalis is a perennial spring-flowering plant that occurs around the Baltic Sea in Scandinavia and in mountainous areas in central and southern Europe (Edqvist 2018, Chappuis 2014). In Sweden the species is Red Listed and classified as highly endangered (EN) since 2010, but of least concern (LC) globally and in Europe (Chappuis 2014, SLU Artdatabanken 2020). However, populations in Europe are declining, especially in Sweden, Denmark and Poland, and some are already extinct (Chappuis 2014).

P. vernalis occurs on sandy, well-drained acidic grassland or heathland, including forested heathland, often in connection to eskers (Grzyl *et al.* 2013, Edqvist 2018). It prefers open habitat without a dense understorey vegetation, such as on grazed land or formerly grazed land (Berglund 2015). The species is thought to have poor dispersal ability, and likely depends on perturbations, such as fire or soil disturbance for survival (Edqvist 2018). *P. vernalis* usually grows in tight clusters of leaf rosettes, here referred to as 'tufts'. There is disagreement regarding whether a *P. vernalis* tuft consists of a single plant, or several individuals close together due to seeds germinating close to the maternal plant (Stridh & Rehnberg 2014, M. Aronsson, M. Edqvist, T. Ferm, B. Stridh, personal communication 2021). Nevertheless, tufts are easily recognised in the field, and recording tufts is therefore the most common method used for *P.vernalis* surveys in Sweden.

Conservation biologists have tried various methods to increase populations of *P. vernalis* including sowing seeds and planting seedlings (Betz *et al*. 2013, Hildingsson 2021), thinning forest cover (Karlsson & Svensson 2019) and burning (Sandström *et al.* 2014, 2017). There are no studies on the Conservation Evidence database (www.conservationevidence.com) specifically for *P. vernalis*, but *Pulsatilla vulgaris*, a more common relative of *P. vernalis*, has been shown to benefit from removal of vegetation and leaf litter before seed sowing (Piqueray *et al.* 2013). Other studies,

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not included in the database, have shown that *P. vernalis* benefits from sowing seeds and planting seedlings, as both the germinating seed and young seedlings seem to be particularly vulnerable (Betz *et al*. 2013, Hildingsson 2021). Thinning of forest has also been shown to benefit *P. vernalis* (Karlsson & Svensson 2019).

Naturally occurring forest fires have declined sharply in Fennoscandia in the past century (Wallenius 2011) and this is likely to have had a detrimental effect on *P. vernalis*. Prescribed burning of forests has positive effects on vascular plant richness, and on diversity of understorey plants, but the effects are highly variable across studies and ecological situations, and burning may be harmful to certain taxa (Eales *et al.* 2018; Agra *et al.* 2020). Studies have shown that *P. vernalis* thrives in the years after a forest fire, when most other competing vegetation has not had time to re-establish (Sandström *et al.* 2014, 2017). *P. vernalis* seeds also benefit from mineral soil exposed after burning (Ljung 2018). Therefore, prescribed burning has been used to support populations of *P. vernalis*. Sandström *et al*. (2017) compiled data from Swedish case studies in a meta-analysis and found that burning had a positive effect on the number of *P. vernalis* tufts during the first year after burning. They also found that a combination of burning and mechanical disturbance, such as raking, had a positive effect on *P. vernalis*, whereas mechanical disturbance alone had no effect. In the meta-analysis by Sandström *et al.* (2017), only eight case studies included prescribed burning, and only two case studies included data three years after the fire.

Here we present results from an unplanned experiment testing the effects of burning on the population of *P. vernalis* in open and forested heathland in eastern Sweden. The County Administrative Board of Uppsala, Sweden carried out a prescribed burning in 2018 at an existing *P. vernalis* locality on Marma military training area in Uppsala County. We combine previously collected data before the fire (2016) with surveys after the fire (2020, 2021) using identical survey methods, to quantify the effects of prescribed burning on the *P. vernalis* population two and three years after the fire. We compare the data from the burned areas with nearby unburned control sites to determine whether the prescribed burning affected the number of tufts and number of flower stalks of *P. vernalis*.

ACTION

Prescribed burning

The study site at Marma military training area (Figure 1), located near Marma village outside Älvkarleby in eastern Sweden (60°30′56.2″N, 17°26′48.6″E) is an area of open and forested heathland (mainly young pine trees) on sandy soil.

Figure 1. Geographical location of Marma military training area, near Marma village outside Älvkarleby in Sweden. Background map © Lantmäteriet.

Part of this study area was burned on 11th of May 2018 by a team from the County Administrative Board of Uppsala, as a conservation action to benefit the population of *P. vernalis*. The entire study area (Figure 2a) was scheduled to be burned in 2018, but due to very windy conditions on the day, burning was stopped after only half the area was burned. The unburned parts of the heathland and forested heathland therefore formed a natural control. The burn was shallow due to the high soil moisture content expected in spring; only the upper soil layer was burned, but vegetation such as heather and shrubs were burned down. In addition to the planned burning in 2018 there are also occasional small fires in the heathland areas, but not in the two forested areas. These small fires are a side effect of the Armed Forces' live-firing exercises when the vegetation is dry. They are quickly extinguished and have occurred in the area since the opening of the firing range in 1883 (Eriksson *et al.* 2005). These smaller fires occur randomly in both the burned and unburned control areas of our study and, as such,

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we have not included them in our analysis. However, these occasional small fires have likely reduced the overall amount of vegetation in both our open heathland areas compared to similar completely unmanaged open heathland areas outside the live firing range.

We divided the study area into four sub-areas, based on the habitat type and fire treatment (Figure 2a). The heathland area consisted of open vegetation on sandy soil with low cover, of, for example, heather *Calluna vulgaris*, lingonberry *Vaccinium vitis-idaea* and the lichen *Cladonia stellaris*. The heathland area that was burned in 2018 was 1.312 hectares (Figure 2b), and the unburned heathland area (control) was 1.186 hectares (Figure 2c). The forested heathland consisted of young pine *Pinus sylvestris* and spruce *Picea abies* forest, with undergrowth of *V. vitis-idaea,* red-stemmed feathermoss *Pleurozium schreberi* and pine and spruce saplings. The forested heathland area burned in 2018 was 0.320 hectares (Figure 2d), and the unburned forested heathland area (control) was 0.416 hectares (Figure 2e).

Field survey

For many years, parts or all of Marma military training area have been surveyed for *P. vernali*s, and data uploaded to the Swedish open database, Artportalen (Shah & Coulson 2021). In this study we included survey data from 2016, 2020 and 2021 as these were the only years when complete surveys of the entire study area were available. The surveys were completed by the same person (Löfgren: 2016, 2020), or by using the same method as Löfgren (Hagström: 2021). Each year, we surveyed the whole area on foot in May, when *P. vernalis* has clearly visible leaves and (if present) flowers. The number of flower stalks per tuft is believed to indicate the vitality of this long-lived perennial (Stridh *et al.* 2016). At this time of year, most other vegetation is not yet green, so detecting *P.vernalis* is straightforward. We surveyed all four areas in a single day, recording the number of tufts and the number of flower stalks. In this study we defined a tuft as a tight cluster of leaf rosettes with bases no more than 5 cm apart. We counted all tufts and flowers within a 5 m radius of a found tuft (termed an 'aggregation'). Aggregations are used to geolocate subsets of the population for the Artportalen database; we reported the number of tufts, number of flowers, and location for each aggregation to the Artportalen database (Shah & Coulson 2021). One large aggregation containing many tufts of *P. vernalis* was located exactly on the border between the two areas 'heathland + burn' and 'heathland' and was therefore excluded from this study (Figure 2a).

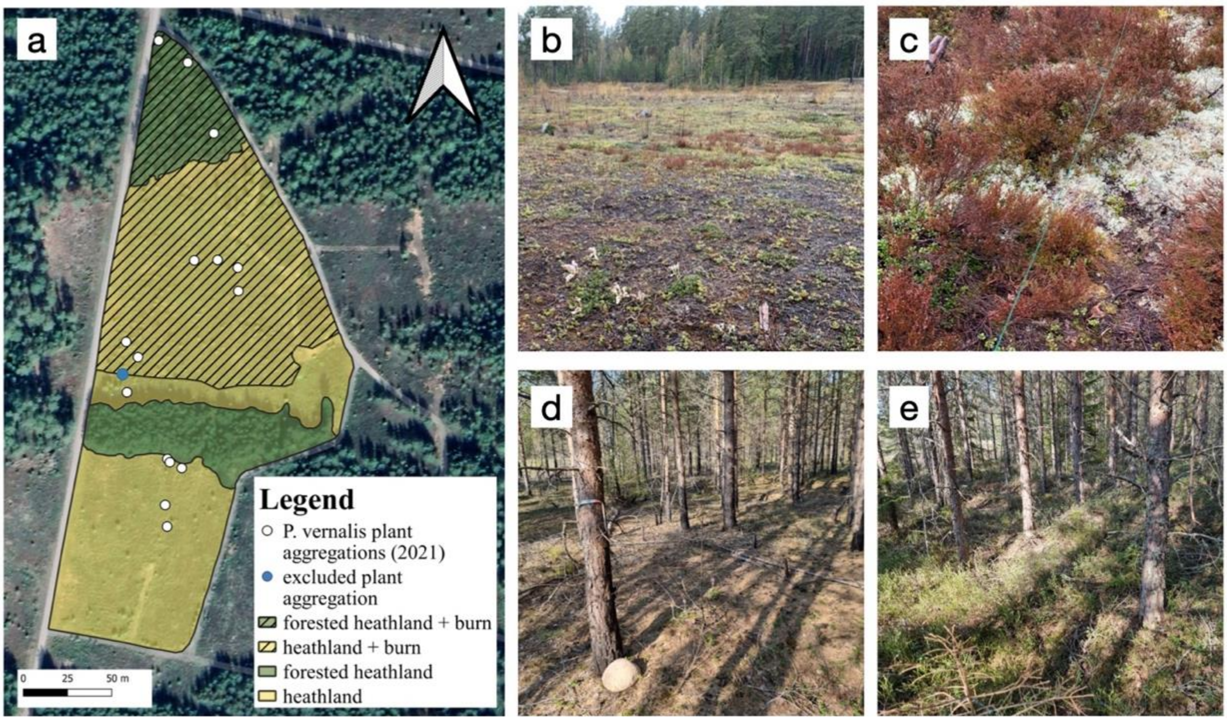

Figure 2. The study area 2021. a) Map over the surveyed area at Marma military training area, Älvkarleby, Sweden with the four subareas indicated. Photos from May 2021 of the b) burned heathland, c) unburned heathland, d) burned forested heathland and e) unburned forested heathland. Background map © Lantmäteriet; photos by C. Hagström (b, c) & T. Löfgren (d, e).

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Statistical analysis

We used Chi-square test to compare the actual distribution of *P. vernalis* tufts each year in the burn and control areas with the expected (based on area). We corrected for the multiple comparisons using sequential Bonferroni corrections. For example, the total heathland area was 2.512 hectares, of which burned heathland was 1.326 ha and control heathland was 1.186 ha. If the number of tufts only depended on area, we would expect the burned heathland to have 52.8% of the tufts, and the control heathland 47.2% of the tufts found in the total heathland area. Similarly, if burning has no effect we would expect the burned forested heathland to have 43.5% of the tufts, and the control forested heathland to have 56.5% of the tufts.

We used Wilcoxon nonparametric tests to determine whether, for each year, burning increased the number of flowers per tuft of *P. vernalis* in the burned area compared with that in the control area. Because the number of flowers had been reported for each aggregation rather than for individual tufts, the average number of flowers per tuft for each aggregation (the number of flowers in a specific aggregation / the number of tufts in that aggregation) was used as a data point. Unfortunately, this means that the number of data points was small, and statistical comparisons were therefore only possible for some of the years. We corrected for the multiple comparisons using Sequential Bonferroni corrections. Analyses were made in SPSS 27 and R version 4.0.2 (R Core Team 2021).

CONSEQUENCES

Overall, in both heathland and forested heathland, the number of *P. vernalis* tufts increased significantly in the burned areas compared to the unburned control areas (Figure 3). In the heathland, in 2016, before the burn, there was a higher number of *P. vernalis* tufts in the control area compared to in the area-to-be-burned than what was expected if they were evenly distributed (X^2 = 16.29, d.f. = 1, p < 0.0001; Table 1, Figure 3a). However, in 2020 two years after the burn, this had flipped and there was a higher number of *P. vernalis* tufts in the burned heathland area compared to the unburned control area than was expected by area size alone (X^2 = 31.88, d.f. = 1, p < 0.0001; Table 1, Figure 3a). In 2021, three years after the burn, the number of *P. vernalis* tufts in the burned heathland area compared to the unburned control was no different from that expected by area size alone (X^2 = 0.20, d.f. = 1 p = 0.65; Table 1, Figure 3a). In the forested heathland areas, in 2016, before the burn, the number of *P. vernalis* tufts in the area-to-be-burned compared to the control was no different from that expected by area size alone (X^2= 0.033, d.f. = 1, p = 0.86; Table 1, Figure 3b). However, in 2020 two years after the burn, there was a higher number of *P. vernalis* tufts in the burned forested heathland area compared to in the unburned control area than what was expected by area size alone (X^2 = 13.22, d.f. = 1, p = 0.0003; Table 1, Figure 3b). Also in 2021, three years after the burn, there was a higher number of *P. vernalis* tufts in the burned forested heathland area compared to in the unburned control area than was expected by area size alone (X^2 = 11.55, d.f. = 1, p = 0.0007; Table 1, Figure 3b).

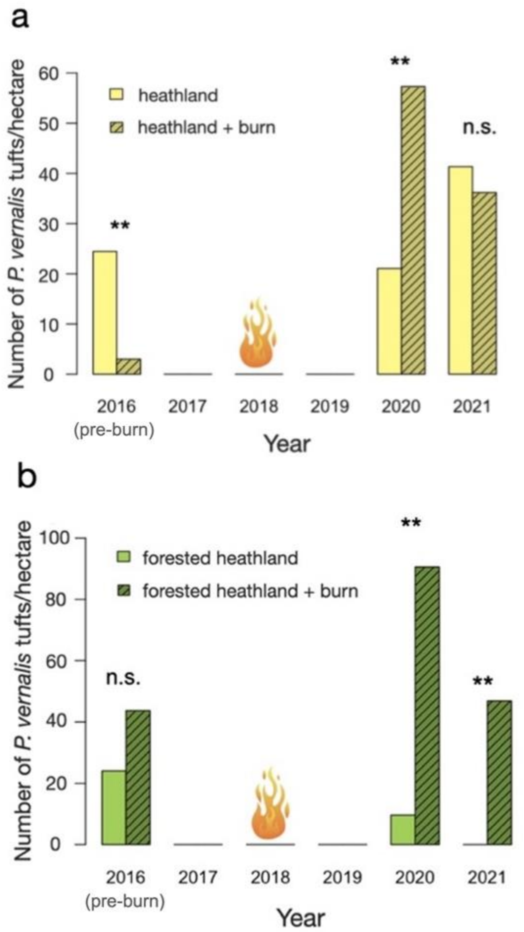

Figure 3. Number of *Pulsatilla vernalis* tufts (plants) per hectare before the burn (2016) and two (2020) and three (2021) years after the burning in 2018, in (a) heathland and (b) forested heathland areas. Note that we show the calculated tufts per hectare to standardize for area and facilitate visual comparisons. Our raw data are presented in Table 1. For each year, we tested the distribution of tufts in the burned and control areas compared to the expected distribution for the size of the respective areas, then used sequential Bonferroni corrections: n.s. = not significant, ** = p < 0.01.

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The number of flower stalks per *P. vernalis* tuft, thought to indicate vitality, also increased in burned areas but not in unburned control areas (Table 1, Figure 4). In the heathland, in 2016 there was no obvious difference in the number of flowers per tuft between the area-to-be-burned and the control area (Table 1, Figure 4a; too few data points for statistical analyses). In 2020, two years after the fire, there were slightly more flowers per tuft in the burned heathland area compared to in the unburned area, although the difference was not statistically significant (Wilcoxon W = 14.0, p = 0.19; Table 1, Figure 4a).

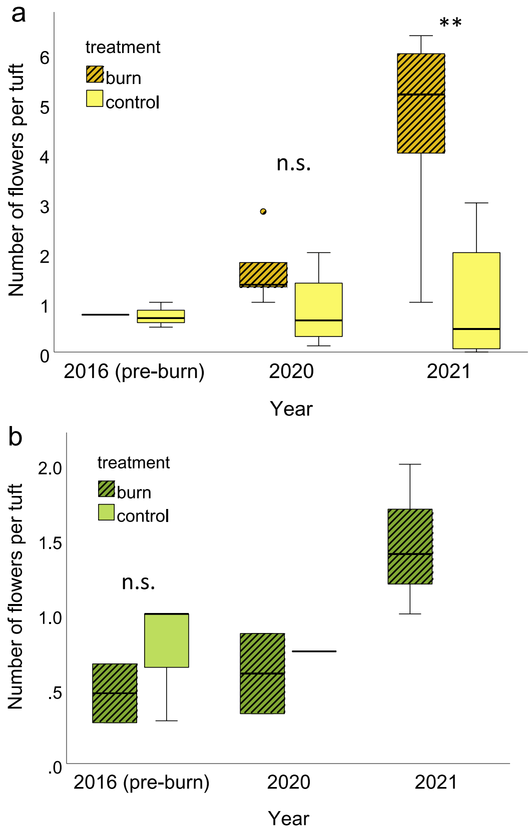

Figure 4. Number of *Pulsatilla vernalis* flowers per tuft before the burn (2016) and 2 (2020) and 3 (2021) years after the prescribed burning in 2018, in (a) heathland and (b) forested heathland areas. The boxplots indicate the median (horizontal line), and the first and third quartiles (box). Note that for a few years there is only a single datapoint (only median shown), or no plants at all, meaning we could not carry out statistical comparisons. For each year, we tested the number of flowers per tuft in the burned area against the number in the control area using Wilcoxon tests, then used sequential Bonferroni corrections: n.s. = not significant, ** = p < 0.01.

In 2021, three years after the fire, there were significantly more flowers per tuft in the burned heathland area compared to in the unburned area, (Wilcoxon W = 23.0, p = 0.009; Table 1, Figure 4a). In the forested areas, in 2016 before the fire there was no significant difference in the number of flowers per tuft between the area-to-be-burned and the control area (Wilcoxon W = 11.0, p = 0.40; Table 1, Figure 4b). After the fire, the number of flowers per tuft increased in the burned forested area, whereas *P. vernalis* could no longer be found in the unburned area (Table 1, Figure 4b; too few datapoints for analyses).

Although we did not quantify it, another likely consequence of the fire was a reduction in the amount of understorey vegetation. In 2021 there was a noticeable difference in the amount of understorey vegetation between burned and unburned areas. In the burned heathland and burned forested heathland there was only sparse growth in the field layer, whereas in the unburned heathland and unburned forested heathland, there was denser growth in the field layer (Figure 2). In the unburned heathland, the vegetation in the field layer consisted mostly of heather *Calluna vulgaris*, lingonberry *Vaccinium vitis-idaea* and *Cladonia stellaris*, whereas in the unburned forested heathland there was mostly *V. vitis-idaea,* red-stemmed feathermoss *Pleurozium schreberi* and new establishments of pine *Pinus* and spruce *Picea* (Figure 2 c,e). There was a denser growth in the field layer in the burned forested heathland compared to the vegetation in the burned heathland (Figure 2 c,d). Similarly, there was denser growth in the field layer in the unburned forested heathland compared to the vegetation in the unburned heathland (Figure 2 c,e).

Costs

The prescribed burning performed in 2018 was funded by the County Administrative Board of Uppsala and the Swedish Armed Forces who own the Marma military training area. Before the burning, one person from the County Administrative Board (16 hours) and two people from the Swedish Armed Forces (8 hours each) worked with preparations including internal planning, informing the fire department, preparing maps and the military tank truck. During the burning, 8 people from the County Administrative Board worked on site (8 hours each). For 48 hours after the burning (after 'last smoke') one person from the Swedish Armed Forces regularly monitored the site (total 16 hours). Travel expenses were considered negligible or included in salary costs, as was use of the military tank truck. With salary estimates based on a standard rate of £38.12/hour, the County Administrative Board funded £3078 and the Swedish Armed Forces £1184. The total cost of the prescribed burning was approximately £4262.

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Table 1. Number of tufts and the mean number of flower stalks per tuft of *P. vernalis* each year of surveying at the four sub-areas of Marma military training area. NA = not applicable, as there were no tufts found this year.

	Number of tufts			**Flower stalks per tuft** Mean (standard error of the mean)		
Area and treatment	**2016 (pre-burn)**	**2020**	**2021**	**2016 (pre-burn)**	**2020**	**2021**
heathland	29	25	49	0.73 (0.15)	0.85 (0.41)	1.00 (0.51)
heathland+ burn	4	76	48	0.75	1.67 (0.32)	4.62 (0.82)
forested heathland	10	4	0	0.76 (0.24)	0.75	*NA*
forested heathland + burn	14	29	15	0.47 (0.20)	0.60 (0.27)	1.47 (0.29)

DISCUSSION

This study shows that on heathland and forested heathland (young pine forest) at Marma military training area, prescribed burning had a positive effect on both the number of *P. vernalis* tufts and the number of flower stalks per tuft, up to three years after the burning. A possible reason for this beneficial effect on *P. vernalis* is the reduction of surrounding dense vegetation and therefore reduction of competition.

The number of *P. vernalis* tufts increased after the burning, both in the burned heathland and in the burned forested heathland, compared to before the prescribed burning. In the heathland, the increase in number of tufts compared to the unburned control area was most noticeable two years after the fire, whereas in the forested heathland the increase in number of tufts compared to the unburned control area was significant both two years and three years after the fire. These results support the earlier findings that prescribed burning increases the number of *P. vernalis* tufts (Sandström *et al*. 2017), although that before-and-after study only found an increase in years one and two after the fire, not in year three.

Our study showed a significant increase in the number of flower stalks per tuft after the burning the heathland, and a similar pattern in the forested heathland. In contrast, the multi-site before-and-after study by Sandström *et al.* (2017) did not find a consistent effect of burning on the number of flowering tufts, seemingly due to large variation in flowering across sites and years. They suggest that this could be driven by variation between sites and years in environmental conditions such as weather. The fact that our experiment included unburned control sites allowed us to disregard effects caused by weather. When studying highly variable and weather-dependent traits such as flowering in *P. vernalis*, it is particularly valuable to be able to include control sites in addition to the temporal comparison. Unfortunately, control sites are rarely considered when conservation actions are planned but including them already in the planning stage facilitates evaluation of conservation actions (Ockendon *et al.* 2021).

A single case study, as presented here, only allows limited statistical analyses and the results should be generalized with caution. Monitoring data on *P. vernalis* is collected annually at many other locations in Sweden by conservation biologists and citizen-scientists (Shah & Coulson 2021), and some of these populations are likely to be the foci of conservation actions. To enable aggregated quantitative analyses of these actions it is important that the survey methods are consistent. This is challenging for *P. vernalis*, as there currently is ambiguity in the national instructions regarding the unit to report to the central database. Whereas some citizen science botanists report the number of tufts (clusters; in Swedish 'tuva') of *P. vernalis*, others report the number of leaf rosettes within a tuft (Shah & Coulson 2021; M. Aronsson, G. Aronsson, M. Edquist, B. Stridh, T. Ferm personal communication). This ambiguity unfortunately makes it difficult to compare data across different collectors, and thus limits its use. Like Sandström *et al.* (2017), we recommend that the main data unit to report for *P. vernalis* should be tuft (Swedish:

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'tuva') as this unit is easy to consistently identify for data collectors.

We conclude that the prescribed burning at Marma military training area in 2018 by the County Administrative Board of Uppsala benefited the population of *P. vernalis* and is an appropriate method for the management of this population. We hope that this case study may stimulate further quantitative evaluations of conservation methods.

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