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Research Article

Monitoring protocols for the evaluation of the impact of wild boar (*Sus scrofa*) rooting on plants and animals in forest ecosystems

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Abstract

The management of wild boar (*Sus scrofa*) is an issue of increasing global conservation concern. Statistically robust monitoring protocols, allowing the detection of biologically relevant changes in biodiversity indices due to wild boar activities, are crucial tools for the management of wild boar populations. The goal of our study was to present a robust procedure targeted towards elaborating monitoring protocols for the evaluation of the impact of wild boar rooting on forest plants and animals.

We compared two pairs of macro-areas characterized by contrasting levels of rooting activity. We then evaluated the effect of rooting on several parameters of four forest communities: understorey vascular plants, ground invertebrates, Carabid beetles and small mammals. We found that the evenness of the Carabid community was significantly higher in high-rooting macro-areas. Moreover, the diversity and evenness indices of understorey vascular plants were higher in high-rooting macro-areas, while the abundance of the Etruscan shrew (*Suncus etruscus*) was higher in the low-rooting macro-areas, although these differences were only marginally significant. The results of the remaining tests were all non-significant. However, confidence intervals of measured effect sizes always included biologically relevant effects; therefore, these results should be considered inconclusive.

The magnitude of the effect we detected on several biodiversity indices was considerably small (probably due to a certain degree of rooting affecting currently and in the past all the macro-areas), therefore high sampling effort should be required to detect such subtle differences. Researchers and practitioners should carefully consider the complexity of monitoring the impact of wild boar and the choice of the parameters to investigate since our study clearly shows that monitoring some biodiversity indices requires a substantial investment of sampling effort and a well-structured a priori-planning phase. Failing to do so will inevitably lead to a waste of resources and /or wrong management decisions.

Introduction

European populations of the wild boar (*Sus scrofa*) are increasing in local abundance and geographic range across Europe (Massei and Genov, 2004; Apollonio et al., 2010). In large part this is due to the life-history traits of the species (an habitat generalist with omnivorous diet and high fertility; Bieber and Ruf 2005; Gethöffer et al. 2007) combined with the lack of predators in the more anthropized environments (Saez-Royuela and Telleria, 1986) and the reintroduction for hunting purposes (Long, 2003; Rollins et al., 2007). Given the high ecological and socio-economic impacts of the species (Pimentel et al., 2000), wild boar management is a growing concern for public administrations and conservation agencies (Bieber and Ruf, 2005; Monaco et al., 2010).

The activities of the wild boar, such as nesting, feeding and rooting, have been suggested to impact a wide array of taxa and ecological processes such as the chemical properties of soil (Mohr et al., 2005; Risch et al., 2010; Wirthner et al., 2011), the forest understorey and grassland diversity and dynamics (Howe et al., 1981; Ickes et al., 2001, 2005; Siemann et al., 2009; Bueno, 2011; Bueno et al., 2011), the plant community structure (Cushman et al., 2004), seedling survival, abundance and distribution (Sweitzer and Van Vuren, 2002; Gómez and Hódar, 2008), acquatic and terrestrial invertebrates communities (Vtorov, 1993; Baubet et al., 2003; Kaller and Kelso, 2006; Giménez-Anaya et al., 2008), the abundance of saprophageous and predatory soil arthropods (Mohr et al., 2005), ground-nesting birds (Rollins and Carroll, 2001; Schaefer, 2004), herpetofauna (Wilcox and Van Vuren, 2009; Jolley et al., 2010) and small mammals (Singer et al., 1984; Focardi et al., 2000).

In addition to the above mentioned negative impacts some positive effects have been detected on tree species regeneration (Lacki and Lancia, 1986; Ickes et al., 2001, 2005; Siemann et al., 2009) and on the species richness of forest understorey (Welander, 1995; Milton et al., 1997). Some authors hypothesized that some of the abovementioned positive effects may be related to the aeration of the soil and the increasing mobilization of nutrients due to rooting (Singer et al., 1984; Lacki and Lancia, 1986). Barrios-Garcia and Ballari (2012) suggested that idiosyncratic results on rooting effects could be due to the species range (native or introduced) where studies were carried out.

Although much important work on the impact of wild boar activities has been carried out, to date most of existing knowledge comes from observation-correlation research. Only a few studies were specifically designed to compare biodiversity patterns in areas with contrasting impacts of wild boar activities. In fact, the ideal study should compare areas with similar habitat types where the species is present and areas where it is not, e.g. fenced vs. unfenced areas. To our knowledge,

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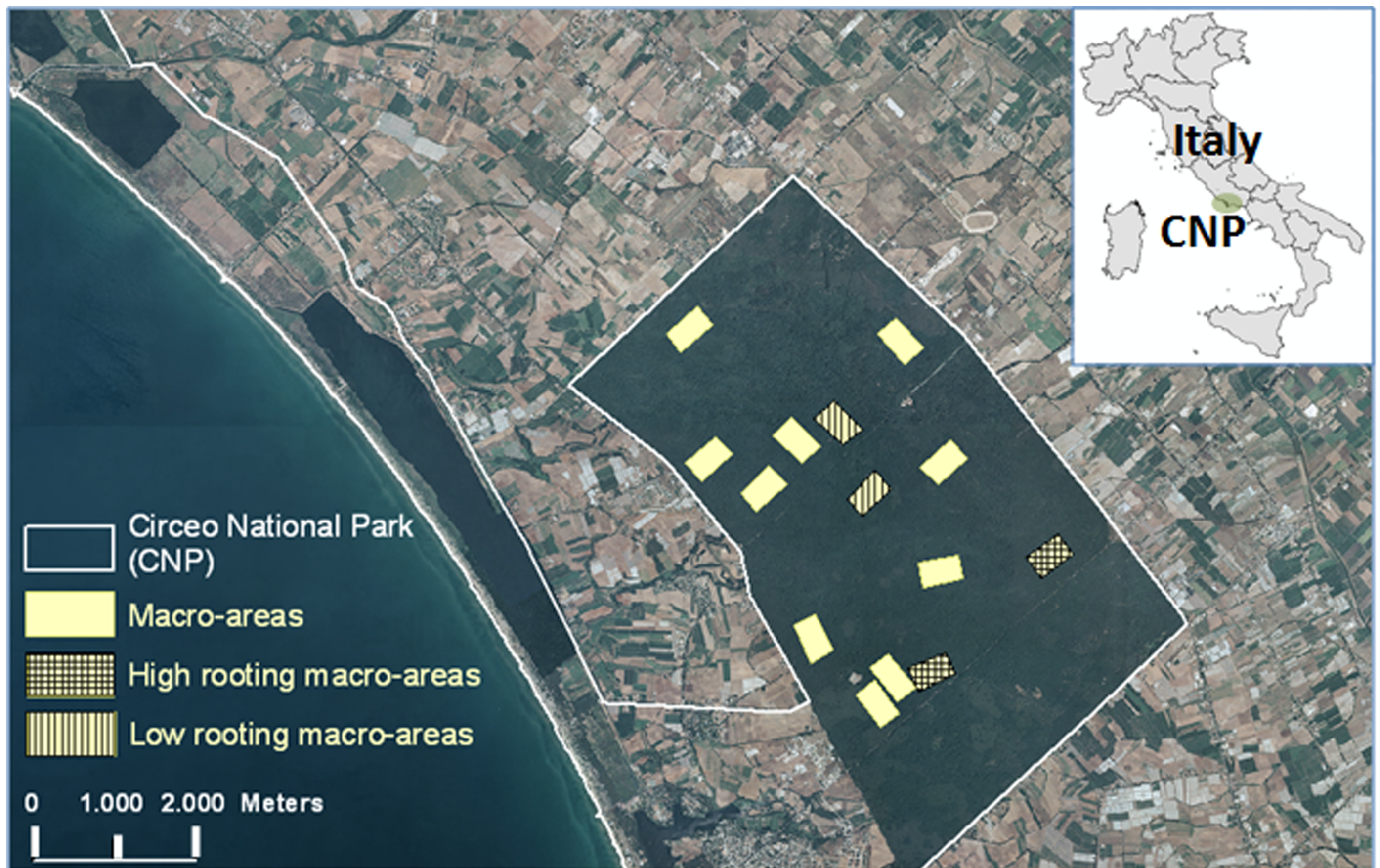

Figure 1 – Study area. The fourteen macro-areas surveyed for rooting levels and the identified two pairs of macro-areas with high-rooting levels and low-rooting levels.

only three European studies have compared fenced and unfenced areas (Mohr et al. 2005; Gómez and Hódar 2008; Risch et al. 2010; Wirthner et al. 2011; the last two publications refer to the same research). Alternatively, researchers should compare areas with significantly different levels of wild boar abundance or rooting intensities.

Study designs as those described above are crucial since they allow to estimate the magnitude of the rooting effect, a fundamental parameter essential to build reliable monitoring programs. In fact, the development of robust monitoring protocols implies the knowledge of a specific parameter that is related to the investigated phenomenon, i.e. the effect size, and the adoption of a rigorous statistical procedure, i.e. the power analysis (Cohen, 1988; Elzinga et al., 2001). The effect size is the absolute difference between populations in the parameter of interest scaled by the within-population standard deviation (Steidl et al., 1997). The power analysis is the tool which allows to determine the sample size needed to be able, with a defined probability (power = $1 - \beta$), to detect the statistical significance (for a given α level) of a specific difference or change in a parameter (i.e. the effect size; Douglas 1999). When statistical tests have low statistical power ($1-\beta$), the risk of making a type II error, i.e. to accept the null hypothesis when it is false, is high (Cohen, 1988). This risk increases as sample size or effect size decreases (see Fig. 1 in Steidl et al. 1997).

It follows that a monitoring program with an inadequate sample size will have a high probability to not detect a biologically relevant change in the parameters investigated, and the risk of failure of such programs is high (Legg and Nagy, 2006). Consequently, monitoring programs not developed on sound and rigorous basis will not guarantee the adoption of the most appropriate management actions and the prevention of waste of human and economical resources. Nevertheless, despite the crucial role of effect sizes and relative confidence intervals (hereafter named CIs) in the development of monitoring protocols, such parameters or data necessary to calculate it (e.g. means and standard deviations), as well as the statistical power of the analysis are rarely estimated or reported (Nakagawa and Cuthill, 2007; Marsh and Trenham, 2008). The main consequence is that we currently lack adequate empirical data and statistical robustness to develop reliable monitoring protocols.

A successful result of a wild boar management program depends on: 1) a quantitative assessment of the studied impact and its magnitude (i.e. is wild boar impact subtle and hard to detect or conspicuous and easy to detect?), 2) the adoption of a robust decision-making procedure to select the most effective management strategies, and 3) the evaluation of the effectiveness of these strategies (VerCauteren et al., 2005). In the present study we aimed to tackle the first and the third of the above aspects.

We compared, through a one level factorial experimental design, two pairs of macro-areas with contrasting intensity of rooting activity (two low-rooting vs. two high-rooting) in the residual lowland forest of the Circeo National Park (Central Italy).

Our aim was to describe a robust procedure to develop monitoring programs of wild boar rooting effects on biodiversity. Our scopes were: 1) to assess the magnitude of the effect of wild boar rooting reduction on i) richness and diversity of understorey vascular plants, ii) richness, diversity and abundance of ground dwellers invertebrates (hereafter named ground invertebrates) and Carabids, iii) abundance of small mammals species; 2) to develop monitoring protocols which allow the detection (at $\alpha = 0.05$ level and with a power = 0.80) of a variation of 20% and 50% in these parameters (e.g. the ability to detect a 20% increase in species richness), applying a step-by-step statistical procedure.

The empirical evaluation of the impact of wild boar rooting on the investigated parameters enabled us to estimate the effects sizes and the relative CIs. The procedure we present allows the development of robust sampling protocols for the evaluation of the efficacy of management actions (e.g. population control or fencing).

Methods

Study area

The study area is located in southern Lazio (Central Italy), within the Circeo National Park (41°20′29″N – 13°02′49″E; Fig. 1). The climate is Mediterranean characterized by hot summers and with winter temperatures which rarely fall below 10° C; rainfall is concentrated mainly in autumn and winter. The forest (a large fragment of residual lowland woodland enclosed in an intensely cultivated landscape), covers an area of about 3200 ha, over a series of slightly undulating late-Pleistocene dunes (between 0 and 50 m a.s.l.). *Quercus cerris* and *Q. frainetto* dominate the most spread forest type.

The last wild boar census in the forest date back to 1989, when a population of 350 individuals were estimated (0.11 ind./ha; Ragni et al. 2005).

Study design

We adopted a study design that would allow us to compare ecological parameters of understorey vascular plants, ground invertebrates, Carabids and small mammal communities between areas characterized by contrasting levels of rooting activity. We preliminarily surveyed the study area with the aim of finding four rectangular macro-areas of 15 ha (500 m long and 300 m large) with statistically different levels of rooting: two macro-areas with relatively low levels of rooting and two macro-areas with relatively high levels of rooting. We adopted the size of 15 ha for two reasons: 1) to reduce the variability in rooting-events among the macro-areas due to localized intense rooting activity; 2) to obtain a macro-area width which allowed us to position three trapping grids not too close to each other. The choice of four final macro-areas for the biodiversity sampling was due to logistical constrains.

In order to determine how many macro-areas we had to sample for rooting-events (see below for details on rooting sampling) to detect such pairs of macro-areas, we carried out a set of *a priori* power analysis (Cohen 1988; for details see Appendix S1). Following the simulation procedure, the required sample size (number of macro-areas per number of linear transects) resulted in being 14 macro-areas. Within each one of the four macro-areas we systematically sampled 12 linear transects (100 m of length), in order to represent even habitat proportion. Such sampling design enabled us to detect, at $\alpha = 0.05$ level, a minimum difference in rooting levels among the macro-areas of 40%, with a power of 0.935. A priori power analysis were carried out with G*Power 3 software (Faul et al., 2007). The rooting-events sampling was performed in three consecutive and sunny days on April 2010.

The rooting levels in the macro-areas was assessed counting rooting signs in five meters wide strips along the transect. Since in our study area rooted patches had mainly a circular or oval shape, we assumed the number of rooting signs as a proxy of the rooted area, i.e. more rooting-events = more rooted area. We defined a single rooting event as a 15 cm-wide sign of rooting activity on the ground; wider signs were evaluated as multiples of the single event (e.g. a sign wide 30-45 cm = two rooting-events, a sign wide 45-60 cm = three rooting-events, and so on; Fig. S1).

Mean rooting signs of each macro-area were analysed through a one-way ANOVA, and statistically significant differences among the macro-areas were detected ($F = 11.5$, $df = 167$, $p < 0.001$; Tab. S1). To identify the pairs of macro-areas significantly different we used the Tahmane post-hoc test. We finally selected four macro-areas with pairwise significantly different levels of rooting for subsequent field sampling (Fig. 1). These macro-areas did not differ in soil properties and overstorey structure (Burrascano et al. *submitted*). In the same macro-areas Burrascano et al. (*submitted*) analysed rooting effects on understorey composition and functional traits. In the interpretation of their results they highlighted that low-rooting macro-areas were subjected to intensive rooting activity following an increase of wild boar population in the last 50 years. Subsequently, a consecutive high spread of spinescent and clonal species in the understorey inhibit wild boar feeding activities in such areas, leading wild boars to move in those areas where we found the higher rooting levels. Comparing the results of Burrascano et al. (*submitted*) with those of Blasi et al. (2002) on the cover of spinescent species in the low rooting areas, we might approximate that rooting intensity in such areas begin to considerably reduce 10-15 years ago. Along with Burrascano et al. (*submitted*) interpretation, for our methodological purposes we considered any difference in the ecological parameters we investigated as a response to a reduction of wild boar rooting pressure. However, we should be cautious in interpreting our results, since in the present research we were unable to control for other factors (such as microclimatic conditions or the abundance of predators) which may influence the parameters we analysed.

Biodiversity sampling

Within each selected macro-area vegetation sampling was carried out in six square plots (side: 20 m) randomly distributed. For each plot we compiled the list of understorey vascular plants and the percentage cover value of each species. Sampling was performed during May-June 2010.

Small mammals were sampled through three trapping grids placed randomly in each macro-area: two grids of pitfalls (obtained from plastic containers 25 cm deep and 12 cm wide) for the sampling of Insectivores; one grid of Longworth boxtraps (Longworth Scientific Instruments, Abingdon, UK) to sample Rodents. We chose grids as sampling units for our study since these are commonly used for long-term monitoring purposes (Elzinga et al., 2001). Each square grid was made of 49 traps (7 transects each of 7 traps) spaced 10 m. Within each boxtrap we placed the nesting material and a bait of sunflower seeds. In the boxtrap grids we performed five trapping sessions, with the exception of one grid in which only three sessions were performed due to logistic constraints. The number of trapping sessions of the pitfall grids varied from three to five. No baits were placed inside the pitfall traps. All traps in each session remained active for five nights and were checked daily during the first hours of the morning. The overall sampling effort was 4410 trap-nights for boxtraps and 7350 trap-nights for pitfall traps. Following species identification Rodents and Insectivorous were weighted, sexed, assigned to an age class (adult, sub-adult, juvenile) and individually marked. Since identification of *Apodemus* species in the field is difficult, a sample of tissue was gathered from individuals belonging to this genus for subsequent laboratory identification through species-specific PCR (Polymerase Chain Reaction; Bugarski-Stanojević et al. 2013).

The sampling of ground invertebrates and Carabids was carried out with the same pitfall traps and with the same timing used for the Insectivores sampling, with the exception of one grid where, for logistical constrains, we sampled the ground invertebrates and Carabids one less trapping session. The pitfall traps were placed with the upper edge on 2-3 cm below the ground level, limiting the capture probability of subterranean species. The overall sampling effort with pitfall traps was 7105 nights-trap. All individuals captured were taken for subsequent laboratory identification. We underline that in each trapping session the four boxtrap grids were activated simultaneously, while the eight pitfall grids were activated four at a time, in ten consecutive days. Small mammals, ground invertebrates and Carabids were sampled on May-August 2010.

Statistical analysis

For each sample unit (macro-area) we calculated: 1) richness, Shannon diversity index and evenness of understorey vascular plants; 2) richness, Shannon diversity index, evenness and an abundance index of ground invertebrates and Carabids; 3) abundance index of Rodents and Insectivores. Due to the different sampling effort among grids, richness and Shannon index of ground invertebrates and Carabids were verified through rarefaction method (Gotelli and Colwell, 2001) by ECOSIM 7.0 (Acquired Intelligence Inc. & Kesey-Bear, available from: http://garyentsminger.com/ecosim/index.htm). The results of the rarefaction method we used (Colwell et al., 2004) showed that richness and Shannon diversity indices of ground invertebrates and Carabids fell outside the range of CIs estimated through the simulation (Tab. S2 and Tab. S3). Therefore, we considered only the individuals captured

in the last three sessions, common to all grids, to calculate these parameters.

Values of computed parameters were grouped (low rooting macro-areas vs. high rooting macro-areas) and tested for significant differences in mean values through the two-tailed t-test. To improve the interpretability of the test results we calculated standardized effect sizes, i.e. Cohen's d (Cohen, 1988),

$$d = \frac{|\text{mean sample A} - \text{mean sample B}|}{\text{within} - \text{population standard deviation}} \tag{1}$$

and 95% CIs for each difference in the parameters (Nakagawa and Cuthill 2007). We followed Cohen (1988) to classify the size of the measured effect: small effect = 0.4, medium effect = 0.6 and large effect = 0.8. In accordance with Steidl et al. (1997), when the result of a t-test was not significant (at α = 0.05 level), if the upper 95% CI of the effect size exceeded a probable biologically significant effect size, we considered the test inconclusive. Following Cohen (1988) definition, we assumed values of d > 0.8 as biologically significant effect sizes. Statistical tests were performed with SPSS software (SPSS Inc., Chicago, IL, USA). Effect sizes and CIs were elaborated through Practical Meta-Analysis Effect Size Calculator (available from: http://www.campbellcollaboration.org/resources/eect_size_input.php).

Monitoring protocols development

For the development of the monitoring protocols, we assumed the detection of 20% and 50% yearly change on each of the investigated parameters (evenness excluded), with data gathering in fixed macro-areas. This means that our monitoring protocol would allow perceiving a moderate (20%) or a large (50%) annual change (decline or increase) in the target ecological parameters. These arbitrary values were chosen in order to provide a range of biologically meaningful changes that was not too extreme (extremely small or extremely large) hence of potential wide interest.

We adopted the following procedure to determine the required sample sizes. First, the absolute difference in the mean values of each parameter between low- and high-rooting macro-areas

$$D = |\text{mean low-rooting} - \text{mean high-rooting}| \tag{2}$$

was transformed in a percentage of the higher mean:

$$D_p = \frac{D}{HM} \times 100 \tag{3}$$

where HM is the higher between low- and high-rooting mean.

Second, in order to obtain the differences in mean values corresponding to 20% and 50% (X_i) we applied a proportion:

$$D : D_p = X_i : i \tag{4}$$

where i = 20 and 50.

Third, we calculated the corresponding effect sizes applying the Cohen's d formula with X_i in the numerator, using in the denominator a pooled standard deviation (Kirk, 1996):

$$SD_{Pooled} = \sqrt{\frac{(N_{HR}-1) \times SD^2_{HR} + (N_{LR}-1) \times SD^2_{LR}}{(N_{HR}-1) + (N_{LR}-1)}} \tag{5}$$

where N_{HR} and N_{LR} are the sample sizes respectively in high- and low-rooting macro-areas, while SD_{HR} and SD_{LR} are the corresponding standard deviations.

In order to determine the optimal sample sizes, the effect sizes obtained from the third step were used to carry out *a priori* power analysis for one-tailed t-test on two dependent means, at α = 0.05 level and power = 0.80. The sample size elaborated for each taxon corresponds

Table 1 – Results of two-tailed t-tests on community and population parameters. For each parameter of understorey vascular plants, ground invertebrates, Carabids and small mammals in the low and high rooting macro-areas, are reported: sample size (N), mean and standard deviation (SD), as well as for each test the degrees of freedom (df), *p* values, effect sizes (*d*) and their 95% CIs. Significant results are in bold.

	Rooting	N	Mean	SD	t	df	*p*	*d*	95% CI
Understorey vascular plants									
Richness	Low	2	17.333	0.471	-0.277	2	0.808	0.277	-1.692–2.247
	High	2	17.5	0.707					
Shannon diversity	Low	2	1.759	0.257	-3.626	2	0.068	3.626	0.439–6.812
	High	2	2.571	0.185					
Shannon evenness	Low	2	0.425	0.069	-3.574	2	0.070	3.574	0.416–6.732
	High	2	0.626	0.040					
Ground invertebrates									
Richness	Low	2	55	0	0.429	2	0.710	0.429	-1.554–2.411
	High	2	52	9.899					
Shannon diversity	Low	2	13.413	0.841	0.984	2	0.429	0.984	-1.091–3.060
	High	2	10.420	4.217					
Shannon evenness	Low	2	0.648	0.016	1.179	2	0.360	1.179	-0.944–3.303
	High	2	0.582	0.077					
Abundance index	Low	2	1.273	0.098	-3.380	2	0.078	3.380	0.326–6.433
	High	2	1.896	0.241					
Carabids									
Richness	Low	2	7.5	0.707	0	2	1	0	-1.96–1.96
	High	2	7.5	4.950					
Shannon diversity	Low	2	1.324	0.022	-0.557	2	0.634	0.557	-1.441–2.554
	High	2	1.572	0.630					
Shannon evenness	Low	2	0.658	0.020	-8.474	2	**0.014**	8.474	2.283–14.664
	High	2	0.827	0.020					
Abundance index	Low	2	0.038	0.004	2.058	2	0.176	2.058	-0.366–4.482
	High	2	0.018	0.013					
Insectivores									
Abundance index	Low	2	0.0004	0	–	–	–	–	–
Crocidura suaveolens	High	2	0	0					
Abundance index	Low	2	0.011	0.001	3.765	2	0.064	3.765	0.502–7.028
Suncus etruscus	High	2	0.003	0.003					
Rodents									
Abundance index	Low	2	0.005	0.006	0.514	2	0.658	0.514	-1.478–2.506
Apodemus flavicollis	High	2	0.003	0.0004					
Abundance index *Apodemus sylvaticus*	Low	2	0.019	0.008	1.217	2	0.348	1.217	-0.917–3.350

to the number of macro-areas to be sampled, with each macro-area including: one 7x7 boxtraps grid for Rodents, two 7x7 pitfalls grids for Insectivores and ground invertebrates and Carabids, and six 400 m^2 square plots for the sampling of understorey vascular plants.

Results

Rooting effects

The results of two-tailed t-test of understorey vascular plants showed that no indices differed significantly among the high rooting macro-areas and the low rooting macro-areas. Only understorey Shannon diversity and understorey Shannon evenness marginally differed (respectively $p = 0.068$ and 0.070), with lower values in the low-rooting macro-areas (Tab. 1). Understorey richness did not differ significantly among the contrasting rooting levels (Tab. 1). Since all upper bounds of 95% CI of vascular plant parameters exceeds values considered biologically significant, the tests should have been considered inconclusive.

The ground invertebrates trapping resulted in the capture of 10531 individuals, where the main part of these belong to the Carabidae family (47.4%), to the Arachnida class (8.4%), and to the order of Hymenoptera and Collembola (respectively 6.1% and 4%). The richness, Shannon diversity and evenness indices of ground invertebrates did not significantly differ among high and low rooting macro-areas (Tab. 1), while the abundance index marginally differed ($p = 0.078$), with higher values in the high rooting macro-areas. The effect size was large for all the indices ($d > 0.8$), apart for species richness that showed a small effect ($d < 0.5$). However, the upper bounds of the 95% CIs of all ground invertebrates indices were high ($d > 2$), indicating that all the tests should be considered inconclusive (Tab. 1 and Fig. 2).

Regarding Carabids, the Shannon evenness index showed significant higher values in the high rooting macro-areas ($p = 0.014$), and the analyses have produced a very large effect size (Tab. 1). The richness of Carabids presented exactly the same value among the high and the low rooting macro-areas, consequently the effect size value was equal to zero (Tab. 1). The other two indices of Carabids, the Shannon diversity and the abundance index, did not differed significantly among the contrasting rooting levels, but while for Shannon diversity the values were on average higher in the high rooting macro-areas, we had on average a higher number of catches in the low rooting macro-areas (Tab. 1). Since the upper bounds of 95% CI of these last two parameters exceeds values considered biologically significant, the tests should have been considered inconclusive.

Regarding Insectivores, we captured individuals of *Crocidura suaveolens* only in the low rooting macro-areas. As a consequence, we did not perform any test for this species. On the contrary the abundance index of *Suncus etruscus*, showed a marginally significant difference ($p = 0.064$; Tab. 1 and Fig. 2).

The abundance indices of the two Rodent species did not significantly differ between low and high rooting macro-areas (Tab. 1), although higher values were always found in low rooting macro-areas. Since the upper bounds of 95% CI of the measured effect sizes of both parameters were very high ($d > 2$; Tab. 1 and Fig. 2), also the tests on these parameters should be considered inconclusive.

Monitoring protocols

The detection of changes in the understorey vascular plants indices require very few macro-areas: for both 20% and 50% changes, species richness requires only one, while Shannon diversity just two macro-areas (Tab. 2).

Also the detection of changes in ground invertebrates indices require a relatively small sample size. The lower optimal sample sizes were for abundance index and species richness, i.e. less than three macro-areas necessary to detect changes (Tab. 2). Instead the number of macro-areas necessary to detect 20% change on Shannon diversity raised to five, while for a 50% change the optimal sample size is still two.

Regarding Carabids the difference between the number of macro-areas necessary to detect the 20% and the 50% changes in the investigated parameters becomes more marked (rispectively seven and two macro-areas). For species richness of Carabids we could not calculate the optimal sample sizes due the parameter was the same both in the high and in the low rooting macro-areas.

The optimal sample sizes for detecting a significant difference in the abundance index of small mammal species resulted low for *Suncus etruscus* (four and two to detect respectively for a 20% and 50% change), medium for *Apodemus sylvaticus* (16 for a 20% change and four for a 50% of changes) and extremely high for *Apodemus flavicollis* (respectively 57 and 10 to detect a 20% and a 50% changes; Tab. 2).

Discussion

Monitoring protocols

Developing robust monitoring programs implies the determination of the optimal sample size required to detect, with acceptable statistical confidence, a biologically significant variation in the parameters of interest. In spite of the several calls for the application of power analysis when defining the sampling effort (Thomas and Juanes, 1996; Steidl et al., 1997), its application is still rare (Steidl et al. 1997, but see Taylor and Gerrodette 1993; Zielinski and Stauffer 1996; Gryska et al. 1997).

Our field study allowed us to empirically evaluate the magnitude of the effect of rooting on several key ecological parameters. Such empirical results are fundamental for guiding future studies and monitoring protocols development. Our results show how the magnitude of difference between high and low rooting macro-areas is highly dependent on the parameter of interest (Tab. 1), varying considerably from big effect sizes (e.g. $d > 3$ for understorey Shannon diversity and evenness indices or for ground invertebrates abundance) to quite moderate or small effect sizes (e.g. $d < 0.5$ for understorey and ground invertebrates richness). This complicates development of monitoring protocols since higher sampling effort is required to detect the latter subtle differences. Researchers and practitioners should carefully consider the complexity of monitoring the impact of wild boar since our study clearly shows that it requires a substantial investment of sampling effort and a well structured *a priori*-planning phase. Failing to do so will inevitably lead to a waste of resources and/or wrong management decisions.

How general are our sampling protocols? Sampling protocols are inevitably case and context specific, therefore the actual number of sampling units required for our study area will likely be different in another environmental context. Furthermore, the sampling effort will depend on the sampling techniques and, of course, on the percent change that managers are willing to detect. Exploring the infinite range of possible protocols is meaningless. The goal of our study was to establish a first field-based development of a monitoring protocol. Our protocols should then be used to guide the initial sampling phase of future work and then be adjusted, in an adaptive monitoring framework (Lindenmayer and Likens, 2009), to allow integration as new information emerges and to meet local requirements following the same procedures we have adopted. Moreover, since wild boar populations might increase rapidly and their impact strongly depend on their densities (Barrios-Garcia and Ballari, 2012), we stress the importance to obtain, during a monitoring phase, an estimate of wild boar abundance, even so more in areas, as our study area, where the last census dated so far in the time (Ragni et al., 2005).

Despite the intrinsic specificity of sampling protocols, we below point out several messages of general importance which emerge from our power analysis (Tab. 2). First, we found that the detection of a 50% rather than a 20% change in the parameters required a number of sampling units ranging from half to more than one-quarter. Second, among the four investigated communities, that of understorey vascular plants is the one requiring the lowest sample sizes (Tab. 2). However, we recall that the sampling techniques adopted for that community (vegetation plots) are relatively different from the techniques used for the sampling of other communities (trapping grids). On the opposite, monitoring the small mammal community requires very large sample sizes (Tab. 2), with the exception of *S. etruscus*. As for ground invertebrates and Carabids, the sample sizes required for the latter guarantee the monitoring of the same parameters of ground invertebrates. We

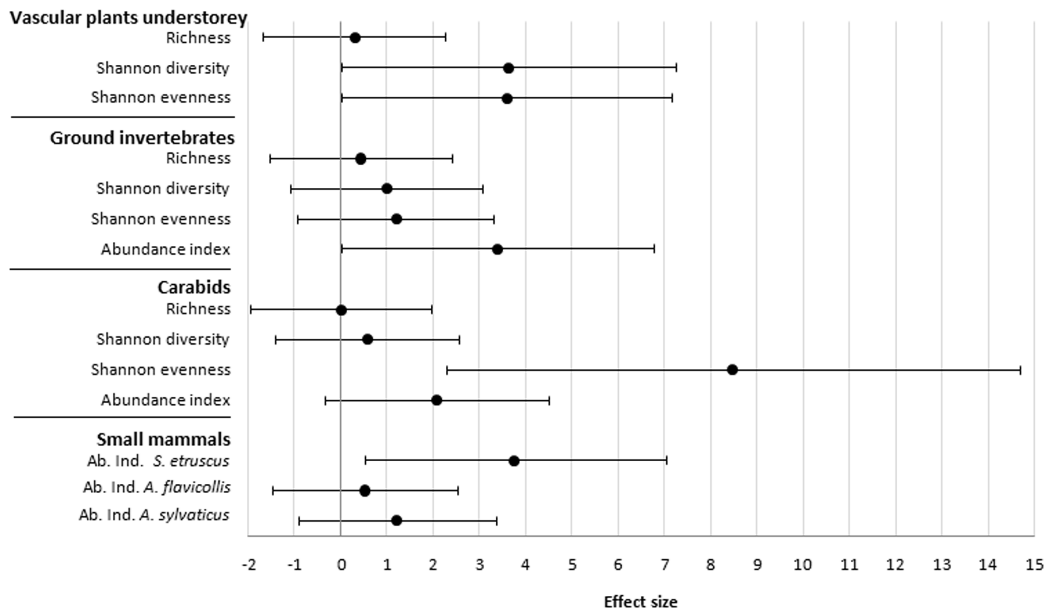

Figure 2 – Effect sizes (points) and 95% Cis (lines) of all the investigated parameters. Ab. Ind.: abundance index.

Table 2 – Effect sizes and required sample sizes to detect 20% and 50% changes. Required sample sizes are expressed in terms of macro-areas (i.e. within each macro-area: six sampling plots for understorey vascular plants, one boxtraps grids for Rodents, two pitfalls grids for Insectivores and ground invertebrates and Carabids).

	Effect Size 20%	Sample Size 20%	Effect Size 50%	Sample Size 50%
Understorey vascular plants				
Richness	5.824	1	14.561	1
Shannon diversity	2.296	2	5.741	2
Shannon evenness	2.226	2	5.565	2
Ground invertebrates				
Richness	1.571	3	3.929	2
Shannon diversity	0.882	5	2.205	2
Shannon evenness	2.321	2	5.803	1
Abundance index	2.058	2	5.144	2
Carabids				
Shannon diversity	0.706	7	1.765	2
Shannon evenness	8.306	1	20.765	–
Abundance index	0.766	7	1.915	2
Insectivores				
Abundance index *S. etruscus*	1.059	4	2.649	2
Rodents				
Abundance index *A. flavicollis*	0.236	57	0.590	10
Abundance index *A. sylvaticus*	0.454	16	1.134	4

suggest that researchers and practitioners should consider these results in helping them target the key-ecological parameters of their study. In addition, the type and shape of sampling units should be carefully considered (i.e. use transects in spite of grids).

We stress that our study was aimed at describing a robust procedure to develop sampling protocols for scientific monitoring programs and not for "managing monitoring protocols" (*sensu* Yoccoz et al. 2001). In fact we did not deal with some issues which, in the decision-making process, could influence the phase of sampling design. In particular, we did not consider the relative importance of Type I and Type II errors (Di Stefano, 2003). In fact, in the protocols development, we set fixed values of α and β (0.05 and 0.2, respectively). This assumption implies that the risk of making a Type I error is four times more important than that of making a Type II error (Steidl et al., 1997). From a conservation perspective this may not always be the case. For instance, in protected areas the aim is to preserve species richness and diversity, and costs of species loss (Type II error) could overcome those deriving from an unnecessary management intervention (Type I error). In the decision-making processes, several procedures were proposed, as the determination of the required sample size for different levels of α and β (Gryska et al., 1997), or the setting of a fixed α:β ratio (Di Stefano, 2003).

Undoubtedly, the selection of the optimal monitoring strategy is strongly dependent on the financial constrains (Joseph et al., 2006). Moreover, the budget available may determine the choice of the biodiversity parameters to be monitored (Yoccoz et al., 2001; Joseph et al., 2006). As an example, in the case of our protocols on small mammals (Tab. 2), the optimal strategy (in the absence of logistical and financial constrains) would be the selection of sample sizes resulting from the abundance index of *A. flavicollis*, which would also permit an effective monitoring of the richness and Shannon index. In a more realistic scenario, due to the great sampling effort required to monitor such parameter, reserve practitioners might face funding constrains which will only allow the monitoring of the abundance index of *S. etruscus*.

Rooting effects

Previous research highlighted that wild boar rooting may have a negative impact on plant species richness (Hone, 2002; Cushman et al., 2004) and diversity (Tierney and Cushman, 2006; Siemann et al., 2009; Bueno, 2011; Bueno et al., 2011), on the abundance of litter-dwelling invertebrates (Vtorov, 1993) and of saprophagous and predatory soil arthropods (Mohr et al., 2005) as well as on the distribution of small mammal species (Singer et al., 1984). Some of our results are consistent with these findings, while other not.

While understorey vascular plant richness of high and low rooting macro-areas was not significantly different, Shannon-Weaver diversity and evenness indices were marginally significantly higher in high-rooting macro-areas. Our results contrasting with previous findings (Tierney and Cushman, 2006; Siemann et al., 2009) are determined by the fact that the lower diversity and evenness of the low-rooting macro-areas are caused by the strong dominance of few species (Burrascano et al., *submitted*). The characteristics of the investigated macro-areas and of the dominant species (*Ruscus aculeatus*) in the low-rooting macro-areas strongly suggest that in these macro-areas the understorey was subjected (in the near past) to relatively intense wild boar feeding activities, and underwent deep compositional and structural modifications that inhibited further feeding activities (further details in Burrascano et al., *submitted*).

We found that the evenness of Carabids and abundance of ground invertebrates were higher, although the latter only marginally, in high-rooting macro-areas (Tab. 1). Most Carabid species are carnivorous and they actively hunt other invertebrates. However, although the abundance index of Carabids was nearly two folds higher in low-rooting macro-areas (Tab. 1), these results were not statistically significant. We thus hypothesize that environmental conditions in low-rooting macro-areas, i.e. a lack of an intensive rooting activiy and a dense understorey layer (Burrascano et al., *submitted*), could have caused a shift in dominance in the Carabid community toward few dominant species. However, we cannot exclude that the differences we found might be determined also by other environmental conditions that we did not considered in our study, i.e. difference in microclimatic conditions, different abundance of predators.

On the other hand, the marginally lower abundance of ground invertebrates in low-rooting macro-areas could be related with the local high abundance of shrews. In fact, besides the fact that we captured individuals of *C. suaveolens* only in these macro-areas, we found that in low-rooting macro-areas the abundance of *S. etruscus* was nearly three times higher (Tab. 1). Although we cannot exclude that such important differences may be correlated with the different abundance of Carabids within the contrasting macro-areas, we argue that an important role could be played again by different habitat conditions. In fact, as noted above, the low-rooting macro-areas are characterized by a well-structured litter layer and a dense cover of a spinescent geophite (*Ruscus aculeatus*), which provide important shelter resources from predation. A similar role seems to be played also on other bulbous geophytes (Burrascano et al., *submitted*).

All other tests resulted in non-significant differences between the contrasting macro-areas (Tab. 1). However, this does not imply that the null hypothesis, i.e. no effect of rooting, has to be accepted blindly. When sample size (or effect size) is small, as in our case (N = 4), the statistical power of statistical tests is low. The consequence is the increase of the risk of making a type II error. Steidl et al. (1997) suggested estimating and analysing measured effect sizes and relative CIs when reporting non-significant results: if a minimum biologically significant effect does not lie outside the CIs, then the null hypothesis cannot be accepted and the test must be considered inconclusive. CIs of our estimates of effect sizes, while including zero values, always included values of effect size that should be considered biologically relevant ($d > 0.8$; Fig. 2). Therefore, due to the small sample size, the results of all non-significant tests may be considered inconclusive.

Furthermore, the lack of significance of the tests conducted on those parameters where we found large effect sizes (e.g. $d > 0.9$ in Shannon diversity index of ground invertebrates) could be attributed to the uncertainty of field data. In particular, we did not deal with detectability of sampled species, which is an important source of data uncertainty (Yoccoz et al., 2001; Tyre et al., 2003). Although we acknowledge the risk to fail in detecting all species in a community survey, we are confident that the high sampling effort we carried out in terms of night-traps significantly reduced this potential bias in our estimates. The above-mentioned lack of significance should be ascribed to the small sample size (number of trapping grids) we adopted, which determined low statistical power of our analysis. We agree with Thomas and Juanes (1996) and Steidl et al. (1997) in stating that statistical power analysis should become a routine procedure in the phase of sampling design and that, when reporting results of significance tests, estimates of effect size and CIs need to be provided.

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Appendix S1. Study design and a priori power analysis.

Table S1. Number of rooting signs collected in each macro-area (descriptive statistics).

Table S2. Individual-based rarefaction method for Ground invertebrates and Carabids richness.

Table S3. Individual-based rarefaction method for Ground invertebrates and Carabids Shannon diversity.

Figure S1. Examples of single (15 cm wide) and multiple (30 cm wide = 2 rooting-events; 45 cm wide = 3 rooting-events) rooting-events detected during the rooting sampling.