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# UNIVERSITÀ DEGLI STUDI DI TORINO

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# Does natural reforestation represent a potential threat to dung beetle diversity in the Alps?

Claudia Tocco\*, Matteo Negro, Antonio Rolando, Claudia Palestrini

Dipartimento di Biologia Animale e dell'Uomo, via Accademia Albertina 13, 10123 Torino, Italy

\* Corresponding author

e-mail: claudia.tocco@me.com, claudia.tocco@unito.it

## Abstract

Traditional agro-pastoral practices are in decline over much of the Alps, resulting in the complete elimination of livestock grazing in some areas. Natural reforestation following pastoral abandonment may represent a significant threat to alpine biodiversity, especially that associated with open habitats. This study presents the first assessment of the potential effects of natural reforestation on dung beetles by exploring the relationships between the beetle community (abundance, diversity, species turnover and assemblage structure) and the vegetation stages of ecological succession following pastoral abandonment. A hierarchical sampling design was used in the montane belt of the Sessera Valley (north-western Italian Alps). Dung beetles were sampled across 16 sampling sites set in four habitat types corresponding to four different successional stages (pasture, shrub, pioneer forest and beech forest) at two altitudinal levels.

The two habitats at the extremes of the ecological succession, i.e. pasture and beech forest, had the greatest effect on the structure of local dung beetle assemblages. Overall, dung beetle abundance was greater in beech forest, whereas species richness, Shannon diversity and taxonomic diversity were significantly higher in pasture, hence suggesting this latter habitat can be considered as a *key conservation habitat*. Forests and pastures shared a lower number of species than the other pairs of habitats (i.e. species turnover between these two habitats was the highest). The two intermediate seral stages, i.e. shrub and pioneer forest, showed low dung beetle abundance and diversity values. Local dung beetle assemblages were also dependent on season and altitude; early-arriving species were typical of pastures of high elevation, whereas latearriving species were typical of beech forests.

It is likely that grazing in the Alps will continue to decrease in the future leading to replacement of open habitats by forest. This study suggests therefore that, at least in the montane belt, reforestation may have potentially profound and negative effects on dung beetle diversity. Maintaining traditional pastoral activities appears to be the most promising approach to preserve open habitats and adjacent beech forests, resulting in the conservation of species of both habitats.

# Keywords

Pastoral abandonment, Scarabaeidae, ecological succession, Generalized linear models, Multivariate Regression Trees, IndVal.

#### **INTRODUCTION**

The European Alps, due to their complex geo-morphological history and the consistent impact of human presence, host a wide range of different habitats and climatic conditions over a relatively small spatial scale. Such environmental heterogeneity is probably one of the main reason why the Alpine chain houses so many species and hence represents for many animal taxa a biodiversity hotspot (Meyers et al. 2000; CIPRA 2008). Pastoral activities, that were practiced for at least six thousand years (Lichtenberger 1994), contributed significantly in shaping the landscape and affecting biodiversity, especially below the tree line (Chemini and Rizzoli 2003; Fisher et al 2008). The farming system of the Alps evolved slowly until the second half of the 20th Century, when the development of industry caused profound socio-economic changes that lead to the overall decline of traditional agro-pastoral systems (Mottet et al. 2006; Chauchard et al. 2007). Mountain areas had become less economically competitive due their isolation and physically disadvantaged conditions (MacDonald et al. 2000). The general shift of human populations towards the valley bottoms and the subsequent abandonment of marginal areas resulted in decline of forest resource utilization, agriculture and traditional cattle-breeding. This situation is now quite evident across a wide range of alpine valleys in Europe (MacDonald et al. 2000) where the decline of traditional agro-pastoral practices, with the complete elimination of grazing, is the main determinant of natural reforestation. After the abandonment of pasture, vegetation succession develops to shrub, pioneer forest and local climax forest, therefore causing the disappearance of open habitats (Tasser and Tappeiner 2002; Chemini and Rizzoli 2003; Tasser et al. 2007). Several studies have confirmed the importance of traditional pastoral practices in preserving open areas and maintaining animal diversity (birds: Laiolo et al. 2004; grasshoppers and butterflies: Marini et al. 2009; bats: Obrist et al. 2011).

Dung beetles (Coleoptera: Scarabaeoidea) are undoubtedly one of the most typical and ecologically relevant arthropod groups in grazed alpine habitats. They have been widely studied during the last twenty years and there now exists a significant body of knowledge on the species' biology and distribution patterns (Verdù et al. 2011). It is well known, for instance, that local

distribution may depend on specific habitat preferences (Barbero et al. 1999; Macagno and Palestrini 2009; Negro et al. 2011) and, in mountain ecosystems, on altitude (Jay-Robert et al. 1997; 2008a; Errouissi et al. 2004; Negro et al. 2011). Furthermore, dung beetles display graded responses to a variety of ecological factors or anthropogenic disturbances (Halffter and Arellano 2002; Spector 2006; Numa et al. 2009) and have been successfully used to evaluate habitat perturbation (Quintero and Roslin 2005; Barragán et al. 2011). As a consequence, they have recently been proposed as a candidate taxon in applied biodiversity conservation research (Nichols and Gardner 2011).

Scarabaeoidea beetles warrant high conservation priority because their feeding and nesting behaviour contribute to a suite of important ecosystem services in that they: i) play a key role in the burying and removal of dung; ii) are responsible for the relocation of faecal material into the soil, so enhancing soil fertility; iii) serve to control the abundance of dung-breeding flies and dung-dispersed nematodes and protozoa and iv) increase porosity, aeration and water infiltration (Spector 2006, Slade et al. 2007, Nichols et al. 2008; Sholtz 2009, Brown et al. 2010).

Changes in land use may have severe consequences for dung beetle assemblages (Verdù et al. 2007; Barragàn et al. 2011; Negro et al. 2011). Natural reforestation following pastoral abandonment, in particular, may represent a significant threat to alpine dung beetle diversity because most species are associated with open habitats (Zamora et al. 2007; Jay-Robert at al. 2008b; Macagno and Palestrini 2009). About 40% of Finnish dung beetle species should now be considered as threatened because of the loss of open habitats due to pastoral abandonment (Roslin and Viljanen 2011).

This study focuses for the first time upon the potential effects of natural reforestation by exploring the relationships between dung beetle assemblages and the vegetation stages of ecological succession following pastoral abandonment at the level of the montane belt, i.e. the lowest belt of mountain forest, occupied mainly by beech *Fagus sylvatica* in the Alps (Shoumatoff and Shoumatoff 2001). The study was carried out in the Sessera Valley, an Italian alpine valley which epitomizes the process of natural reforestation following pastoral abandonment, a situation typical of large areas of the Alps. Due to a major expansion of the wool industry around the beginning of last century, local pastoral activity and forest resource utilization had ceased to be the main source of income for the human population of the valley (Neiretti et al. 2000). Pastoral activities therefore started to decrease and, as a consequence of abandonment, seral stages followed each other with varying rates depending on local conditions. Reforestation is known to be largely

controlled by altitude. Tasser et al. (2007) observed that the velocity of this process at altitudes corresponding to the altitudinal range considered in the present study (1000-1600 m a.s.l), varies from 15 to 30 years. This explains why the landscape of Sessera Valley has been completely modified in just over a century. In the middle of last century one third of Sessera Valley was used as pasture and the remainder was covered mainly by beech forests (Ubertalli and Biasetti 1997). Nowadays all successional stages are still present across the valley, which is largely covered by forests (24.3% beech forest and 19.9% pioneer forests), shrubs (11.8%) and, to a lesser extent, pastures (8.4%) (Gottero et al. 2007).

We considered the assemblages of dung beetles of the valley and analyzed diversity and abundance differences across three seral stages (pasture, shrub, pioneer forest) and the climax habitat (beech forest).

The aim of our work was to evaluate the potential effect of reforestation in the Alps under the likely future scenario in which open habitats are largely replaced by forests.

### **MATERIALS AND METHODS**

#### Study area and sampling design

Dung beetle assemblages were sampled in the Sessera Valley (Fig. 1) in the western Italian Alps (45° 40′ 16″N; 8° 05′ 07″E, Biella, Piedmont), which has been declared a Site of Community Importance ).

The climate is temperate sub-alpine with a mean annual temperature of 10°C and a mean annual rainfall of 1800 mm. The area, characterized by high cloudiness with frequent rainfall during the summer, presents favorable conditions for the development of mesophyllic deciduous forests (IPLA 2003).

Four vegetation types, corresponding to four stages of the ecological succession following pastoral abandonment, were identified: pasture (dominated by graminaceous plants), shrub (alpen rose *Rhododendron ferrugineum* L. and bilberry *Vaccinium myrtillus* L.), pioneer forest (dominated mainly by European white birch *Betulla alba* L. and common hazel *Corylus avellana* L.) and beech (*Fagus sylvatica* L.) forest (namely, the *Luzulo-Fagetum* phytosociological association).

The presence of livestock was restricted to a small farm with about 40 cattle grazing in the nearby pastures. Some wild ungulates (roe deer *Capreolus capreolus*, Linneaus 1758 and red deer *Cervus elaphus*, Linneaus 1758) occurr in the study area.

To evaluate the relative effect of altitude and habitat type (i.e. the four successional stages) on local dung beetle diversity, we considered two different altitudinal ranges (1000-1100 and 1400–1600 m a.s.l.) and, within each altitudinal level, two sampling sites for each habitat type. The hierarchical sampling design was therefore made of 16 sampling sites distributed throughout the 4 habitat types (two replicates in each habitat type) at 2 altitudinal levels.

#### Sampling methods

Five pitfall traps of the hang-bait type (Palestrini et al. 2008) were positioned at each sampling site: four at the corners of a 20x20m square and one at its centre.

Each trap consisted of a 1 liter clear plastic bottle, 9cm in diameter, cut at about 20cm: the top was cut to make a funnel which was inserted into the main 25cm container. The bait, 200g of fresh cow dung, was suspended in gauze on a tripod made with 3 sticks 50cm in length, placed over the trap, close to the entrance of the funnel. A standard mixture of water, liquid soap (to reduce surface tension) and sodium chloride was used as a preserving fluid.

The trapping period lasted from early June to late September 2010, corresponding to the activity period of adult dung beetles in Sessera Valley. All traps were emptied and re-baited every 3 weeks, giving rise to 5 different sampling periods.

Beetles were identified to species level using dichotomous keys (Paulian and Baraud 1982; Dellacasa and Dellacasa 2006). Extraction and study of the epipharynx and/or aedeagus was necessary in the case of poorly distinguishable species.

#### Data analysis

#### Species richness estimators and diversity profiles.

A Completeness analysis of sampling for each habitat type for each altitudinal level was conducted by pooling the two replicates of each habitat (i.e. the individuals sampled in the 10 pitfall traps of each habitat of a given altitudinal level were considered together) using the abundance-based richness estimator (ACE). The inventory of completeness was measured as the percentage of the total number of species predicted by the estimator that were actually recorded. The Rényi diversity profile technique was used to rank communities from low to high diversity (Kindt et al. 2006). Rényi diversity profile values (H<sub> $\alpha$ </sub>) are calculated from the frequencies of each

component species (proportional abundances  $p_i$  = abundance of species i/total abundance) and a scale parameter ( $\alpha$ ) ranging from zero to infinity (Tóthmérész 1995; Legendre and Legendre 1998) as:

$$H_{\alpha} = \frac{\ln(\sum p_i)}{1 - \alpha}$$

It can be demonstrated that values of the Rényi profile at the respective scales ( $\alpha$ ) of 0, 1 and 2 are related respectively to species richness, the Shannon entropy and the Simpson diversity index (Legendre and Legendre 1998; Shaw 2003).

Community A is more diverse than a community B if the diversity profile for community A is above the diversity profile for community B at each any value of  $\alpha$  (Kindt et al. 2006). Communities that have intersecting profiles cannot be arranged in order of diversity. The values of the series for the various use-groups were calculated for these scales of  $\alpha$ : 0, 0.25, 0.5, 1, 2 and 4.

#### Differences between habitats and altitudes

To test for differences in diversity between habitats, altitudes and sampling period, we calculated abundance (N), species richness (S), taxonomic diversity ( $\Delta$ ), average taxonomic distinctness ( $\Delta$ <sup>+</sup>) and taxonomic distinctness ( $\Delta$ <sup>+</sup>) for each trap (i.e. the single trap was the sampling unit). Such taxonomic indices quantify diversity as relatedness of the species within a sample, attempting to capture phylogenetic diversity rather than simple richness of species. According to Clarke and Warwick (1998), taxonomic relatedness is more closely linked to functional diversity and, given their statistical properties (mainly robustness to sampling variation and independence from sampling effort) and their sensitivity to environmental degradation, they may represent useful parameters in terms of bio-diagnostic purposes (Clarke and Warwick 1999; 2001). Taxonomic diversity ( $\Delta$ ) combines taxonomic relatedness with the evenness properties of the abundance distribution. Taxonomic distinctness ( $\Delta$ <sup>\*</sup>) is defined as a measure of pure taxonomic relatedness, being the average path length between any two randomly chosen individuals within a sample. Average taxonomic distinctness ( $\Delta$ <sup>+</sup>) represents the case of taxonomic distinctness ( $\Delta$ <sup>\*</sup>) when calculated on presence/absence data only (Clarke and Warwick, 1998).

Generalized linear models (GLM) were used to test for differences in the dependent variable (N, S,  $\Delta$ ,  $\Delta$ + and  $\Delta$ \*) between habitats. For these analyses, three categorical factors (habitat type,

altitude and sampling periods) were considered. The best distribution to describe abundance and species richness was assumed to be the Poisson (Agresti 1996), while for taxonomic indexes ( $\Delta$ ,  $\Delta^*$  and  $\Delta^+$ ) the best distribution assumed was Gamma (Zuur et al. 2009). Parameters were related to explanatory variables via a logarithmic link function (McIntyre and Lavorel 1994).

Significance tests were performed using the Wald statistic (Dobson 1990). Moreover, the habitat, altitude and sampling period factor estimates, their standard errors and individual statistical significance were explored. In the parameter estimation analysis, pasture, lower altitudinal range (1000-1100 m a.s.l.) and the fifth sampling period were used as the reference categories.

#### Species turnover

Species turnover between habitats was described using a ternary plot (Koleff et al. 2003). We considered the presence/absence of species in a focal habitat compared with each neighbouring habitat in turn. The number of species that are present in both habitats is the pairwise matching component *a*. The number of species that are present only in the neighbouring habitat is *b*, while the number present only in the focal habitat is *c*. The total number of species for the pair of habitats is therefore a + b + c; in percentage terms notation becomes a' + b' + c' = 100%. The original equations for the measures of beta Whittaker diversity (Whittaker 1960; Magurran 1988) have also been re-expressed in terms of *a*, *b* and *c* (Krebs 1999; Koleff et al. 2003). Beta Whittaker diversity was calculated for each pair of habitats.

*Multivariate regression trees and IndVal.* The variation in assemblage structure between habitat types and altitudinal levels and sampling periods was evaluated using Multivariate Regression Trees (MRT; De'ath 2002). This method is an extension of univariate regression trees with multivariate responses. MRT identifies groups defined by environmental variables. The result is presented as a tree of dichotomies. Each dichotomy is chosen to minimize the dissimilarity within each branch. The size of the tree was selected based on the minimum tree size that fell below 1 S.E. of the minimum cross-validation estimate (Breiman et al. 1984; De'ath 2002).

We combined MRT (using the trap as sampling unit) with Indicator Value calculations (IndVal; Dufrêne and Legendre 1997) for the species falling into the MRT branches.

The Indicator Values are at a maximum (=100) when all individuals of a species are found in all samples within a given group, but are completely absent from all the other groups. The statistical significance of the maximum indicator value was evaluated by a randomized re-sampling technique (Monte Carlo randomization with 1000 permutations).

We carried out all the analyses using R 2.1.0 with *vegan*, *mvpart* and *labdsv* packages (R Development Core Team 2005)

### RESULTS

### Species richness estimators and diversity profiles

A total of 27 species (6954 individuals) of three families (Aphodiidae, Scarabeidae and Geotrupidae) were collected (Table 1) during the five sampling periods.

As most of the expected species were caught at each site (completeness ranging from 79 to 100%), it was assumed that the sampling effort was sufficient for correctly describing local assemblages of dung beetles (Table 1). On average, the community as a whole was dominated by Aphodiidae (65.6% of the sampled individuals, 66.7% of the species collected), followed by Geotrupidae and Scarabaeidae (26.1 and 8.3% of individuals and 14.8 and 18.5% of species, respectively). Dominant species were *Anoplotrupes stercorosus, Acrossus rufipes* and *Parammoecius corvinus* (1432, 1180 and 1080 individuals, respectively).

The two intermediate seral stages, i.e. shrub and pioneer forest, were characterized by lower species richness and abundance. The comparison between pasture and beech forest showed that, irrespective of the altitude considered, species richness and diversity were higher in pasture, whereas abundance (total numbers) was higher in beech forest (Table 1).

Rényi diversity profiles confirmed that diversity of shrub and pioneer forest was always the lowest irrespective of the scale of  $\alpha$ , whereas the diversity of pasture was the highest for values of  $\alpha < 4$ , thus confirming that diversity values related to species richness and Shannon diversity (and also Simpson's diversity) were higher in the pasture.

#### Differences between habitats, altitudes and sampling period

*Habitat.* Abundance (N), species richness (S), taxonomic diversity ( $\Delta$ ), average taxonomic distinctness ( $\Delta$ +) and taxonomic distinctness ( $\Delta$ \*) significantly differed among habitats (P < 0.001).

Habitat estimates, in particular, showed that abundance and species richness in pasture (set as a reference category) were significantly higher than those associated with shrub and pioneer forest (Table 2). Moreover, abundance in pasture, but not species richness, was significantly lower than

abundance in forest. Taxonomic diversity ( $\Delta$ ), average taxonomic distinctness ( $\Delta$ +) and taxonomic distinctness ( $\Delta$ \*) associated with pasture were always higher that those associated with shrub, pioneer forest and forest (Table 2 and Fig.2).

Altitude. N and  $\Delta^*$  also significantly differed between the two altitudinal levels (respectively P < 0.001 and P < 0.01). Abundance and taxonomic distinctness ( $\Delta^*$ ) associated with the lower altitudinal range (1000-1100m; set as a reference category) were respectively significantly higher (N: Est. = 0.30, Wald stat = 149.62, P < 0.001) and lower ( $\Delta^*$ : Est. = -0.08, Wald stat = 7.61, P < 0.006) than those of the upper altitudinal range (1400-1600 m).

*Sampling period*. In addition to altitude and habitat, all parameters considered (N, S,  $\Delta$ ,  $\Delta$ + and  $\Delta$ \*) also significantly differed among sampling periods (P<0.001).

The interaction effect between habitat type and sampling period, assessed by means of a two-way ANOVA (to approach normality N and S were previously square-root transformed), was significant only for abundance (P < 0.001).

## Species turnover

The ternary graph showed that the forest/pasture pair shared a lower number of species (46,2%) than the others pairs of habitats (Fig. 3). The maximum number of shared species occurred between intermediate seral stages (pioneer forest *vs* shrub). This pattern was confirmed by beta Whittaker, with high turnover between forest and pasture ( $\beta w = 0.37$ ) and low turnover between pioneer forest and shrub ( $\beta w = 0.13$ ).

#### Multivariate regression trees and IndVal

The first dichotomy in MRT separated the first sampling period from the other four periods (Fig. 4). This reflects a strong asymmetry in the seasonal abundance distribution of dung beetles, most of which (i.e. 53.2 % of individuals) were collected at the beginning of June. The branch of the first period showed two successive nodes regarding habitat type (pasture *vs*. the remaining habitats) and elevation (pasture assemblages are split into high and low elevation assemblages). The branch of the second period had only one node regarding habitat type (forest *vs*. the remaining habitats). The associated Indicator Species Analysis (IndVal procedure), showed that seven first period/pasture species preferred higher elevations, whereas three second period species preferred beech forest (Fig. 4). All of the species that were indicators of beech forest were Aphodiidae (*Limarus zenkeri, Acrossus rufipes, Parammoecius corvinus*), while the seven species

which were indicators of pasture were representative of all three families collected (Aphodiidae: *Colobopterus erraticus, Teuchestes fossor, Aphodius fimetarius*; Geotrupidae: *Geotrupes stercorarius, Anoplotrupes stercorosus*; Scarabaeidae: *Onthophagus fracticornis, Onthophagus joannae*). Summing up, this analysis revealed that seasonality was a relevant structural characteristic of local dung beetle assemblages. Several early-arriving species are typical pasture species of high elevation, whereas some late-arriving species are typical of beech forests.

Our results clearly demonstrated that the two habitats at the extreme of ecological succession, i.e. pasture and beech forest, had the major effect on the structure of local dung beetle assemblages. Overall, dung beetle abundance was significantly higher in beech forest than in any other habitat type, whereas species richness, Shannon diversity and all taxonomic diversity indexes ( $\Delta$ ,  $\Delta$ + and  $\Delta^*$ ) were higher on pasture. The two habitats shared few species: in particular, out of a total of 26 species found in pasture and beech forest, only 12 were in common, giving rise to an high specific turnover. The two intermediate seral stages, i.e. shrub and pioneer forest, showed low dung beetle abundance and diversity. These two habitat types, despite their different vertical vegetation structure, were rather similar in terms of species composition, and were in fact characterized by low turnover. Our results suggest both habitats may be considered as suboptimal to local dung beetles, in keeping with several studies carried out in Mediterranean (Romero-Alcaraz and Avila 2000; Numa et al. 2009; 2012) and alpine (Negro et al. 2011) ecosystems in southern Europe which identified shrub as a suboptimal habitat. Habitat preferences, which have been found in numerous study areas (Barbero et al. 1999; Romero-Alcaraz and Avila 2000; Macagno and Palestrini 2009; Numa et al. 2009; Negro et al. 2011; Verdú et al. 2011), may depend on several ecological factors (Hanski and Cambefort 1991). It has been suggested, in particular, that dung beetles are insensitive to vegetation structure per se, but very sensitive to climatic conditions relating to the structure of vegetation (Martin-Piera et al. 1992; Jay-Robert et al. 2008b).

Habitat type a part, assemblages of the Sessera Valley were significantly conditioned by seasonality and altitude. The first dichotomy in MRT separated the first sampling period from the other four periods because most of the individuals sampled were indeed collected early, at the beginning of June. In Sessera Valley, seasonality and habitat selection seem to be strictly connected. Species of all three families arrived early at pastures (in the first days of June), probably because this habitat type, being open, was the first to provide a sufficient amount of solar heat to provide the energy adults need to start their daily activities. The impression in the

field was that dung beetles, because of a mild spring, had become active rather early, when cattle had not yet arrived. Hence, despite the presence of the dung of wild ungulates, they were hungry and quickly arrived at the first available experimental cattle dung in large numbers (many beetles were observed arriving at the fresh dung before it was even prepared and placed over the trap). By contrast, some species of the family Aphodiidae, which are adapted to cold climates, arrived late in the beech forest because this habitat in summer was cooler and wetter than the others. Seasonal responses of dung beetles are well known and are often considered to be an important interspecific segregation factor which facilitates species (Galante et al. 1995; Palestrini et al. 1995; Errouissi et al. 2009; Zamora et al. 2007; Jay-Robert et al. 2008a).

The importance of seasonality in the different habitats were confirmed by means of two-way ANOVA. We pointed out a significant interaction between habitat type and sampling period for the abundance, but not for the species richness. This reflects the well known phenology of dung beetles, which are poorly active (and are therefore scarcely sampled) in the forest respect the pasture, during the first part of the sampling season.

Altitude was also important in shaping the structure of local assemblages. Abundance at low elevation was significantly higher than at high elevation, and the reverse pattern was true for taxonomic distinctness. Moreover, early pasture species were split according to the altitude they preferred (low *vs.* high elevation). Several studies have shown that altitude is an important factor influencing dung beetle community parameters (Martìn-Piera et al. 1992; Menédez and Gutiérrez 1996; Romero-Alcaraz and Ávila, 2000; Jay-Robert et al. 2008a; Negro et al. 2011). In our study area, the altitudinal gradient was rather narrow because sampling sites were within a range of only 600 metres. This suggests that alpine dung beetles are environmentally fine-tuned and are able to respond to minor altitudinal (and climatic) variations.

It may be acknowledged that other factors, in addition habitat type, seasonality and altitude, may influence dung beetle assemblages. In particular, the spatial configuration and the connectivity of the landscape around each site may have the highest power to explain dung beetle abundance (Numa et al. 2012). However, in our study the influence of landscape factors is likely marginal due to the small size of the study area (Fig.1).

The distribution of wild and domestic ungulates may also potentially influence dung beetle diversity patterns. As for wildlife, it has been demonstrated that the availability of trophic resources produced by native ungulate species have a low influence in the habitat selection of dung beetles in areas with homogeneous distribution of wild ungulates (Numa et al. 2012); this is

just the case of the Sessera Valley where wild ungulates are homogeneously distributed (IPLA 2003). As for livestock, it cannot be excluded that the historical presence of livestock in pastures, and the high quantity of dung there, may have contributed to the increase of diversity of these habitats. A few papers, in fact, have demonstrated the influence of the quantity of dung on species richness (Kadiri et al 1997, Lobo et al. 2006).

#### Conservation implications

This study has demonstrated that most local dung beetle species positively selected pastures, whereas a few other species preferred beech forests. Climax beech forests and pastures shared few species and, accordingly, to preserve local dung beetle assemblages both habitats should be maintained. Pastures, in particular, are crucial for conservation purposes because they are characterized by higher species richness, Shannon diversity and taxonomic diversity values; they can therefore be considered as a key conservation habitat .Dung beetles of pastures, through their feeding and nesting behaviour, are the major contributors to the suite of important ecosystem services which have been shortly outlined in the introduction. Consider, for instance, that several species indicative of the local high altitude pastures (i.e. Anoplotrupes stercorosus, Geotrupes stercorarius, Onthophagus fracticornis and O. joannae) are tunneling or paracoprid species, i.e. they dig tunnels in the soil beneath the dung and carry fragments of dung to the blind ends of those tunnels, hence significantly contributing to dung removal, enhancing soil fertility and increasing porosity, aeration and water infiltration ratios (Brown et al. 2010; Nichols et al. 2008). The importance of alpine pastures of the montane belt in securing dung beetle diversity by comparisons with beech forests is in keeping with the results of previous research (Macagno and Palestrini 2009).

Reforestation by pastoral abandonment is a double-sided threat for open habitat dung beetle specialists because it produces both habitat loss and food resource decrease. Pastoral activities in the Alps have been practiced for at least six thousand years (Lichtenberger 1994) during which dung supply from domestic animals likely remained widely available. During this time, it is likely that alpine dung beetles progressively adapted to this kind of dung. At present, albeit a few specis showed clear preferences for deer dung (*Melinopterus consputus, Otophorus haemorrhoidalis*) or horse dung (*Onthophagus fracticornis*) (Domont et al. 2007), most alpine dung beetles use dung of sheep and Jay-Robert et al. (2008b) showed that wild ungulate manure is not enough to ensure

conservation of dung beetles assemblages. It can be therefore hypothesized that the disappearance of dung of domestic ungulates following pastoral abandonment may impact severely on conservation of dung beetle diversity.

Considering all of the above, our main conclusion is that the effect of reforestation processes in the Alps, by forecasting a future ecological context in which local open habitats in the montane belt will be largely replaced by forests, is potentially detrimental for dung beetles. Maintaining the traditional pastoral activities appears to be the most promising approach to preserve open habitats and adjacent beech forests, resulting in the conservation of species of both habitats. Traditional, low intensity pastoralism also promotes environmental heterogeneity, which is crucial to dung beetle conservation (Barbero et al. 1999; Macagno and Palestrini 2009; Negro et al. 2011).

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Altitude		1000	- 1100		1400 - 1600					
Habitat	Pasture	Shrub	Pioneer forest	Beech forest	Pasture	Shrub	Pioneer forest	Beech forest		
Geotrupidae Latreille, 1802										
Anoplotrupes stercorosus (Scriba, 1796)	274	233	49	245	323	70	30	208		
Geotrupes spiniger (Masham, 1802)	0	1	0	0	0	0	0	0		
Geotrupes stercorarius (Linnaeus, 1768)	23	6	1	2	173	33	63	22		
Trypocopris pyrenaeus (Heer, 1841)	23	8	22	0	3	0	5	0		
Aphodiidae Leach,1815			0	0	0	0	0	0		
Acrossus depressus (Kugelann, 1792)	14	23	19	90	39	29	64	266		
Acrossus rufipes (Linnaeus, 1758)	146	144	89	229	42	170	27	333		
Agolinus satyrus (Reitter, 1892)	0	0	0	0	0	0	0	2		
Agrilinus convexus (Erichson, 1848)	7	0	1	1	17	21	4	5		
Ammoecius brevis (Erichson, 1848)	0	0	0	0	1	1	0	0		
Aphodius fimetarius (Linnaeus, 1758)	0	0	0	0	7	1	1	1		
Bodilopsis rufa (Moll, 1782)	154	11	17	33	6	11	4	20		
Colobopterus erraticus (Linnaeus, 1758)	1	0	0	0	111	0	0	0		
Esymus pusillus (Herbst, 1789)	0	0	0	0	0	0	1	2		
Limarus zenkeri (Germar, 1813)	14	10	51	191	1	10	8	430		
Nimbus contaminatus (Herbst 1783)	1	0	0	0	20	0	0	7		
Nimbus johnsoni (Baraud, 1976)	0	0	0	3	0	0	0	0		
Otophorus haemorrhoidalis (Linnaeus, 1758)	0	0	0	0	1	0	0	0		
Oxyomus sylvestris (Scopoli, 1763)	0	0	0	0	0	0	0	1		
Parammoecius corvinus (Erichson, 1848)	15	97	330	103	27	43	76	389		
Planolinus borealis (Gyllenhall, 1827)	17	17	68	106	3	3	27	18		
Rhodaphodius foetens (Fabricius, 1787)	4	1	0	0	21	0	0	0		
Teuchestes fossor (Linnaeus, 1758)	87	0	3	0	160	2	30	0		
Scarabaeidae Latreille,1802			0	0	0	0	0	0		
Eounticellus fulvus (Goeze, 1777)	0	0	0	0	1	0	0	0		
Onthophagus fracticornis (Preyssler, 1790)	23	0	0	1	430	9	24	59		
Onthophagus joannae (Goljan, 1953)	11	1	0	0	14	1	1	0		
Onthophagus opacicollis (Reitter, 1892)	0	0	0	0	1	0	0	0		
Onthophagus taurus (Schreber, 1759)	0	0	0	0	0	0	0	1		
Total number (N)	814	552	650	1004	1401	404	365	1764		
Shannon-Diversity	7,15	4,74	4,98	6,30	7,49	6,27	9,03	6,44		
Evenness	0,71	0,63	0,67	0,77	0,66	0,70	0,81	0,67		
Simpson's Dominance	0,20	0,28	0,30	0,18	0,18	0,23	0,13	0,18		
Species richness	16	12	11	11	21	14	15	16		
ACE	18,23	15,09	14,67	12,79	27,98	17,94	17,54	19,46		
Completeness ACE (%)	87.76	79.54	75.00	86.03	75.06	78.05	85.52	82.20		

**Table 1**: Number of individuals and species of dung beetles collected for each habitat at the two altitudinal levels considered. Shannon diversity, Evenness, Simpson's Dominance, total number, observed richness and ACE estimate of expected richness are also provided. Inventory completeness is observed richness as a percentage of total expected richness. In this analyses, for need of clarity and synthesis, all individuals sampled in the two replicates of each habitat were

pooled (i.e. the individuals sampled in the 10 pitfall traps of each habitat of a given altitudinal level were considered together).

	Ν		S		Δ*			Δ+			Δ				
	Distribution: POISSON			Distribution: POISSON			Distribution: GAMMA			Distribution: GAMMA			Distribution: GAMMA		
Habitat type	Est.	Wald	p	Est.	Wald	р	Est.	Wald	p	Est.	Wald	p	Est.	Wald	р
Beech forest	0.15	27.56	<0.001	0.09	1.74	N.S.	-0.21	30.16	<0.001	-0.18	26.73	<0.001	-0.21	11.07	<0.001
Pioneer forest	-0.84	489.46	<0.001	-0.37	20.05	<0.001	-0.20	25.61	<0.001	-0.17	19.98	<0.001	-0.16	6.09	<0.01
Shrub	-0.92	566.96	<0.001	-0.48	33.06	<0.001	-0.12	9.08	<0.01	-0.12	9.44	<0.01	-0.24	13.32	<0.001

**Table 2**: Habitat factor estimates and statistical significance (GLZ) for abundance (N), species richness (S), taxonomic distinctness ( $\Delta^*$ ), average taxonomic distinctness ( $\Delta^+$ ) and taxonomic diversity ( $\Delta$ ) parameters. In this analysis pasture habitat type is set as reference category. Significant comparisons are in bold type. In this parameter estimation analysis, pasture lower altitudinal range (1000-1100 m a.s.l.) were used as the reference categories.



**Fig. 1**: Illustration of the study site (3) located in the Sessera Valley (2), North-Western Italian Alps (Biella province, Piedmont) (1). Sixteen sampling sites, with five pitfall traps each, were distributed throughout the four habitat types. Sites were located at two different altitudinal ranges: 1000-1100 m a.s.l. (dashed-line polygon) and 1400-1600 m a.s.l. (solid-line polygon).



**Fig. 2**: Mean taxonomic distinctness ( $\Delta^*$ ) and average taxonomic distinctness ( $\Delta^+$ ) of dung beetles /trap in pasture, shrub, pioneer forest and beech forest. Bars are ± standard errors. Note that GLM analyses made clear that taxonomic diversity ( $\Delta$ ), average taxonomic distinctness ( $\Delta^+$ ) and taxonomic distinctness ( $\Delta^*$ ) associated with pasture were always significantly higher than those associated with shrub, pioneer forest and also beech forest.



**Fig. 3**: Ternary plot representing species turnover between pairs of habitats. Triangle vertices are as follows: a' is the percentage of species that are present in both habitats, b' is the percentage of species that are present only in the neighbouring habitat (the second of the pair), while c' is the percentage of species present only in the focal habitat (the first of the pair). The b  $\beta$  value of Whittaker index is given in brackets.



**Fig. 4**: Multivariate Regression Tree for dung beetle abundances. Numbers below each end branch give the number of traps. Only species which are significantly associated with one of the branches are shown. Statistical significance were obtained by Monte Carlo randomization test (1,000 runs): \* P<0.05; \*\*P<0.01; \*\*\*P<0.001. The model has error term= 0.639 and cross-validated error=0.736 (0.109 SE). P = pasture, S = shrub, PF = pioneer forest, BF = beech forest.