



Article Patterns of Co-occurrence of Rare and Threatened Species in Winter Arable Plant Communities of Italy

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Abstract: Detecting patterns of species co-occurrence is among the main tasks of plant community ecology. Arable plant communities are important elements of agroecosystems, because they support plant and animal biodiversity and provide ecosystem services. These plant communities are shaped by both agricultural and environmental drivers. The pressure of intensive agriculture worldwide has caused the decline of many characteristic arable species and communities. Italy is the European country where arable plant biodiversity is the best preserved. In this study, we assessed the patterns of co-occurrence of rare and threatened arable plants in 106 plots of winter arable vegetation located from Piedmont to Calabria, in the mainland part of the country. For this purpose, we based our investigation on the analysis of a recently acquired dataset and on the European list of rare and threatened arable plants. We highlight how different species of conservation interest tend to occur in the same community. On the other hand, generalist and more competitive taxa show similar patterns of co-occurrence. We suggest that single species of conservation value could be suitable indicators of a well-preserved community. On the other hand, to be effective, conservation strategies should target the whole community, rather than single species.

Keywords: agroecosystems; arable weeds; biodiversity conservation; community assemblage; floristic composition; segetal vegetation; species coexistence

1. Introduction

The concept of plant community relies on the identification of recurrent patterns of species co-occurrence. These patterns can result from many different, non-mutually exclusive factors: chance, history of speciation and migration, dispersal, environmental factors, and biotic interactions between species [1]. Generalist taxa are supposed to co-occur with many species, while specialist taxa are supposed to co-occur with many species, while specialist taxa are supposed to co-occur with relatively few species across their range. Co-occurrence data are useful for defining the diversity of habitats or species niche width without the need to make assumptions on the definition of a habitat or to measure unknown (and often unknowable) environmental factors that influence species distribution [2,3]. Thus, detecting patterns of species co-occurrence is one of the main tasks of plant community ecology and is required in order to understand the drivers that shape extant species assemblages. With regard to arable plant communities (i.e., the weed communities of arable land), very few studies have dealt with species co-occurrence and all have been conducted on a local or regional scale [4–6].

Since agricultural practices started, arable weeds have accompanied crops in their evolution and spread. The selected very specialized arable plant species and communities are totally dependent on agriculture, and are known as "agrestal" or "segetal" [7]. Given that this component of biodiversity competes with crop species, causing possible yield losses, farmers have fought arable vegetation. Nevertheless, many recent studies have proved the importance of arable plant biodiversity in

agroecosystems, not only in environmental terms, but also in agronomic terms. Indeed, the presence of these communities in cultivated fields not only increases plant and animal (especially pollinators) biodiversity, but also provides agronomically useful ecosystem services such as pest regulation and soil protection [8–10]. Species-rich arable plant communities are known to be less harmful to crop production compared to species-poor plant communities that usually host few but very damaging species. Communities rich in species but with low cover, can be maintained by increasing crop diversity, and especially by including crops with different seasonality in the rotation [11]. Thus, floristic richness in arable fields is considered an indicator of both agricultural and environmental sustainability [12].

The species composition of arable plant communities is determined by both agronomic (timing of tillage, preceding crop, control techniques) and geo-environmental factors (elevation, soil type, precipitation) [13–15]. In temperate climates, two very different, broad types of arable plant communities can be distinguished, according to crop seasonality: winter-annual and summer-annual [16–19]. In Europe, winter arable plant communities, such as those that colonize winter wheat, are characterized mainly by native or archaeophyte species, many of which are threatened by agricultural intensification. Some of these species have evolved within crops themselves so that arable fields are their primary habitat and their conservation is totally dependent on extensive agriculture (e.g., Agrostemma githago and Bromus grossus) [20,21]. Summer-annual plant communities are rich in neophytes, cosmopolitan, and generalist taxa [22–24]. This is mainly due to the tropical origin of many species in Europe that behave as summer-annual weeds, and also to some extent, to higher disturbance in summer-annual crops [16,22]. Thus, winter arable vegetation is much more threatened and has a much higher conservation value, to the extent that extensively managed winter arable land was recently recognized as a EUNIS (European Nature Information System) endangered habitat. Italy is very rich in winter arable plants and it is the European country where this habitat best survives, both in extension and conservation status. Therefore, many arable species that are considered threatened on a continental scale are still widespread in Italy, for both natural and socio-economic reasons [15,25,26].

In Europe and in other economically advanced areas of the world, the spread of intensive agricultural practices has caused a relevant decline in typical arable plants that are considered suitable indicators of the conservation status of arable plant communities [27]. The extensive use of herbicides and chemical fertilizers, the shift from crop rotation to monoculture, and seed cleaning have led to floristic changes and the disappearance of many species and communities that were once widespread and characteristic of arable fields and agricultural landscapes [28–30]. In the past few decades, the recognition of the deterioration of arable plant biodiversity has led to the development of conservation strategies that meet the needs of crop production and nature conservation [31–36].

Given the availability of relevant, recent data and the general lack of information on this topic, we investigated the patterns of co-occurrence of rare and threatened arable species across mainland Italy, from the Piedmont region to the Calabria region. The study is part of the ongoing research on arable plant biodiversity in Italy, which so far includes floristic surveys and inventories, vegetation surveys and classification, analysis of geo-environmental gradients affecting arable plant communities, and floristic and ecological shifts over time [15,23,24,30,37]. Our main hypothesis was, as has been highlighted elsewhere, that species of conservation interest tend to occur together in the same community. Such investigations are particularly interesting from the perspective of identifying sustainable management strategies for arable vegetation, both agronomically and environmentally.

2. Materials and Methods

2.1. Study Area and Sampling Design

The dataset consisted of 106 plots of arable vegetation randomly sampled along a marked geo-environmental gradient in mainland Italy, between 45–39° N and 7.5–17° E, in an elevation range of 0 to 1100 m a.s.l. (Figure 1). The dataset was recently used to highlight shifts in floristic composition, species richness and the Shannon diversity of winter arable plant communities along the

main geographic and environmental gradients [15]. The data set is freely available in GBIF (Global Biodiversity Information Facility) as part of a larger database containing species occurrence data from over 1200 floristic and phytosociological relevés [38].



Figure 1. Location of the vegetation plots in Italy and main bioclimatic types (modified from [39]).

The study area is characterized by two main bioclimatic types, Mediterranean and temperate, which are distinguished by the presence and absence of summer drought, respectively. The Mediterranean bioclimate is present in most of the coasts and lowlands of the Peninsula, which amounts to about 40% of Italy. The temperate bioclimate is widespread in most of the north and in the hills and mountains of the Peninsula, i.e., in about 60% of the country (Figure 1). The temperate bioclimatic type also includes a steppic variant in subcontinental areas located in the lower elevations of the Po Plain, and a sub Mediterranean variant, which is found all across low and middle elevations of the Apennines. Mean annual temperatures are higher in southern and western areas, ranging from 10–12 °C in the Po Plain to 18–19 °C on the western coast. Annual precipitation is higher in northern and western areas, and ranges from about 2000 mm in the north-western coastal area to about 500 mm in the south-eastern area [39]. Geological substrates mainly consist of sedimentary rocks such as limestone, flysch, and dolomite. Nevertheless, volcanic and metamorphic rocks are locally present along the western side of the Peninsula. The most common soils are Cambisols, Luvisols, and Regosols. In agricultural areas, neutral or slightly alkaline soils largely predominate over acid soils [40].

The winter-arable biodiversity of Italy is remarkably high, amounting to 987 plant species and subspecies. This is due to both the survival of traditional agriculture and the location of the country in the Mediterranean basin, which is one of the centers of origin of European arable plants [26]. Traditional agricultural areas are still widespread especially in central and southern regions, and notably, in the hilly and mountainous belt. Here, extensively managed winter arable fields host very well-preserved arable plant communities, including many species of conservation interest and characterized by high levels of floristic richness [15].

The data were collected in spring 2018 (from April to June) in fields with winter-annual crops. These were mostly winter cereals (*Avena sativa, Hordeum vulgare, Triticum aestivum, Triticum turgidum* subsp. *durum*), and legumes that are sown in the autumn (*Cicer arietinum, Lathyrus oleraceus* subsp. *oleraceus* -syn. *Pisum sativum* subsp. *sativum-, Trifolium alexandrinum, Trifolium squarrosum, Trigonella foenum-graecum, Vicia sativa*). The arable vegetation was surveyed using one plot per field. Each plot was a strip of 1×16 m, located in the inner part of the field and oriented along seed drill lines. The plot size of 16 m^2 is recommended for surveying European terrestrial herbaceous plant communities [41]. The plot shape in a ratio of 1:16 is able to maximize the number of recorded species among the ones actually occurring, in order to record the full floristic composition of the sampler to move more easily and to avoid serious damage to the crop. In each plot, all of the vascular plant species were recorded and attributed a Braun-Blanquet cover value [43]. The detected plant taxa were identified using the most recent tools available for the flora of Italy [44], and their nomenclature was then updated according to the latest Italian taxonomic standards [45,46].

2.2. Dataset Preparation and Statistical Analyses

All the taxa identified to the genus level, seedlings of woody species and crop species were removed from the dataset. Although plant taxa were identified down to the subspecies level, all the analyses were carried out at the species level. Thus, different subspecies occurring in the same plot were merged into the corresponding species. Due to the difficulties in making a determination to the species level, *Vicia angustifolia* and *V. sativa* were merged under *Vicia sativa* agg., and *Polygonum arenastrum* and *P. aviculare* were merged under *Polygonum aviculare* agg. Abundance data were then transformed into presence/absence data. The result was a 106 plots × 311 taxa matrix. Taxa of conservation interest at a continental level were detected according to the list of the 48 rarest/most threatened European arable plants [34]. The most generalist arable plants were also identified [47]. Finally, nitrophilous species were identified as those having an Ellenberg N value ≥ 7 [48]. We did not take into account the most specialist arable plants in Italy (e.g., *Amaranthus retroflexus, Digitaria sanguinalis, Echinochloa crus-galli*) [47]. These taxa have no conservation value in the study area, and can be considered specialist only in the context of arable fields. Furthermore, our plots were only located in winter arable fields.

The presence/absence matrix was subjected to a species co-occurrence analysis using the function "cooccur()" in the cooccur package of R-project [49,50]. The function applies the probabilistic model of species co-occurrence to a set of species distributed among a set of survey or sampling sites. Starting from community data organized in a species by site matrix, the function returns a list containing pairwise species co-occurrence results. The algorithm calculates the observed and expected frequencies of co-occurrence between each pair of species. The expected frequency is based on the distribution of each species being random and independent of the other species. The analysis returns the probabilities (hypergeometric distribution) that a more extreme (low or high) value of co-occurrence could have been obtained by chance. The "cooccur()" function takes a community dataset of species by site presence-absence data and classifies species pairs as having positive, negative, and random associations, based on the probabilistic model of species co-occurrence from Veech [51]. The species pair combinations that have an expected co-occurrence < 1 are removed from the analysis. If a species

pair is not classified as positive or negative, then it could be truly randomly distributed or unclassifiable due to low statistical power. Truly random associations are those that do not deviate from their expected co-occurrences by more than $0.1 \times$ the total number of sites (default value used in this study, but any proportion can be specified to be more or less strict). The function also returns a list containing a data frame of all pairwise species combinations and of their probability of co-occurring more or less frequently than expected by their observed frequency. Compared to other techniques, the advantage of cooccur is the additional calculation of the probability that species co-occur less than expected [49].

3. Results

Of the possible 48,828 species pair combinations, 45,370 (92.9% of the total) were removed before the analysis because the expected species co-occurrence was <1. Thus, 3458 pairs were analyzed. The cooccur analysis revealed 337 non-random associations between species, representing 9.7% of the analyzed pairs. Of these, 260 were positive associations and 77 were negative associations (Figure 2). The taxa with the highest number of positive associations were *Legousia speculum-veneris* (30), *Galium tricornutum* (26), and *Vicia sativa* agg. (24). Those with the highest number of negative associations were *Polygonum aviculare* agg. (22), *Fumaria officinalis* (7), and *Matricaria chamomilla* (5).



Figure 2. Heat map showing the positive and negative associations determined by the probabilistic co-occurrence model for the arable plant species from our dataset.

Table 1. Positive and negative associations of the rare or threatened arable species present in our dataset based on the co-occurrence analysis. Rare and threatened species [34] are highlighted in bold; the most generalist arable species [47] are underlined; nitrophilous species (Ellenberg $n \ge 7$) are marked with an asterisk [48].

| Rare and Threatened Taxa | Positively Associated Taxa | Negatively Associated Taxa |
|---------------------------|---|--|
| Adonis annua | Aphanes arvensis; Bifora testiculata; Bupleurum subovatum; Coronilla scorpioides; Cota altissima; Delphinium consolida; Euphorbia falcata; Galium tricornutum ; <u>Geranium dissectum;</u> Gladiolus italicus; Legousia speculum-veneris ; Medicago polymorpha; Muscari comosum; <u>Ranunculus arvensis</u> ; Scandix pecten-veneris; Vicia sativa agg.; Vicia lutea | <u>Poa annua</u> *; Polygonum aviculare agg.; <u>Sonchus oleraceus</u> * |
| Agrostemma githago | Galium tricornutum | Helminthotheca echioides; Lysimachia arvensis |
| Bifora radians | Galium tricornutum | |
| Centaurea cyanus | Veronica arvensis; Viola arvensis | Veronica persica |
| Filago pyramidata | Legousia speculum-veneris | |
| Galium tricornutum | Adonis annua; Agrostemma githago; Bifora radians; Bifora testiculata; Buglossoides arvensis; Bupleurum subovatum; Carduus pycnocephalus; Convolvulus arvensis; Coronilla scorpioides; Euphorbia falcata; Gladiolus italicus; Lathyrus aphaca; Lathyrus ochrus; Legousia speculum-veneris; Medicago arabica; Medicago polymorpha; Muscari comosum; <u>Ranunculus arvensis</u> ; Rhagadiolus stellatus; Scandix pecten-veneris; Sonchus asper*; Torilis nodosa; Trigonella sulcata; Valerianella eriocarpa; Vicia sativa agg.; Vicia lutea | Epilobium tetragonum; <u>Poa annua</u> *; Polygonum aviculare agg.; <u>Ranunculus sardous;</u> <u>Veronica persica</u> |
| Lathyrus aphaca | Alopecurus myosuroides*; Galium tricornutum; Legousia speculum-veneris; Ranunculus arvensis; Scandix pecten-veneris | |
| Legousia speculum-veneris | Adonis annua; Anthemis cotula; Avena sterilis; Bifora testiculata; Bupleurum subovatum; Chondrilla juncea; Convolvulus arvensis; Coronilla scorpioides; Cota altissima; Crepis sancta; Delphinium consolida; Euphorbia falcata; Fallopia convolvulus; Filago pyramidata; Galium tricornutum; Gladiolus italicus; Glebionis segetum; Lathyrus aphaca; Lathyrus ochrus; Medicago polymorpha; Muscari comosum; Myosotis arvensis; Ranunculus arvensis; Rhagadiolus stellatus; Scandix pecten-veneris; Stachys annua; Tordylium apulum; Trigonella sulcata; Vicia lutea; <u>Viola arvensis</u> | <u>Poa annua</u> * |
| Lolium temulentum | Scandix pecten-veneris | |
| Ranunculus arvensis | Adonis annua; Anthemis cotula; Bifora testiculata; Bupleurum subovatum; Convolvulus arvensis; Coronilla scorpioides; Delphinium consolida; Euphorbia falcata; Fallopia convolvulus; <u>Galium tricornutum;</u> Helminthotheca echioides; Lathyrus aphaca; Lathyrus ochrus; Legousia speculum-veneris; Medicago polymorpha; Rhagadiolus stellatus; Scandix pecten-veneris; Stachy annua; Trigonella sulcata; Valerianella eriocarpa; Vicia lutea | Glebionis segetum; Lolium multiflorum; <u>Poa annua</u> *; <u>Viola arvensis</u> |
| Scandix pecten-veneris | Adonis annua; Aphanes arvensis; Bifora testiculata; Buglossoides arvensis; Bupleurum subovatum; Coronilla scorpioides; Delphinium consolida; Euphorbia falcata; Galium tricornutum; Gladiolus italicus; Medicago arabica; Medicago polymorpha; Muscari comosum; Lathyrus aphaca; Lathyrus ochrus; Legousia speculum-veneris; Lolium temulentum; Ranunculus arvensis; Rhagadiolus stellatus; Sherardia arvensis; Trigonella sulcata; Vicia lutea; Vicia sativa agg. | Chenopodium album*; <u>Matricaria chamomilla;</u> Polygonum aviculare agg.; Veronica persica |

There were 23 rare and threatened arable species present in our dataset: Adonis aestivalis, Adonis annua, Adonis flammea, Agrostemma githago, Ajuga chamaepitys, Asperula arvensis, Bifora radians, Bromus arvensis, Bromus secalinus, Caucalis platycarpos, Centaurea cyanus, Filago pyramidata, Galium tricornutum, Gypsophila vaccaria, Kickxia elatine, Lathyrus aphaca, Legousia hybrida, Legousia speculum-veneris, Lolium temulentum, Neslia paniculata, Ranunculus arvensis, Scandix pecten-veneris, and Turgenia latifolia. Of these, 11 were non-randomly associated with other species and all of them had at least one positive association with another rare and threatened arable species, with the exception of *Centaurea cyanus*. Negative associations of species of conservation interest were mostly with species listed among the most generalist ones (Table 1).

Similarly, generalist species showed patterns of co-occurrence. For instance, the ubiquitous neophyte *Veronica persica* was positively associated with the highly generalist *Poa annua* and *Stellaria media*. *Sonchus oleraceus* co-occurred instead with *Chenopodium album, Lysimachia arvensis*, and *Polygonum aviculare* agg., all generalist and highly competitive taxa.

The only species from our dataset to be listed as both of conservation interest and among the most generalist weeds was *Ranunculus arvensis*. Nevertheless, our results showed that in the study area this taxon is positively associated mostly with other species of conservation interest, and negatively associated with generalist taxa.

4. Discussion

The highlighted patterns of co-occurrence showed that, in the study area, arable species of conservation value at a European level tend to occur together in plant communities. On the other hand, generalist taxa can also be found to be associated in arable land. Thus, where a species of conservation interest is found, it is more likely to be located in a well-preserved community. Conversely, the occurrence of a generalist species increases the probability of finding a simplified and deteriorated community. These findings suggest the potential use of rare and threatened arable species as indicators of the quality of arable plant communities. They also suggest that every action aimed at the preservation of arable plant biodiversity should target communities as a whole, rather than each single threatened taxon. In the context of arable vegetation, the proportion of occurring rare and typical arable species has already been suggested as a useful parameter to assess the conservation status of arable communities. This is especially true when compared to assessments simply based on species richness and on Shannon or Simpson diversity [27]. If, on the one hand, a species-rich community is intrinsically more valuable from a conservation point of view, is balanced, and sustainable, then, on the other hand, the ingression of generalist and nitrophilous species is one of the main causes of the decline of arable plant communities [52]. This suggests that species-richness on its own is not enough to make evaluations on conservation value. For instance, summer-annual arable plant communities show higher α and β -diversity than winter-annual ones in central Europe, but are characterized by a higher proportion of neophyte alien species [16]. Consequently, a community that hosts less species, but of higher conservation value, should be considered better preserved than a species-richer community that hosts many generalist taxa.

It should be noted that the present study was conducted on a limited dataset, though in principle it is representative of the variability of winter arable plant communities in Italy because of the wide distribution of the used vegetation plots, both in space and in different environmental contexts. Although there is lot more data available from the literature, these were mostly obtained through phytosociological surveys carried out using the classic Zurich-Montpellier approach [43]. This approach uses preferential sampling with non-standardized plot sizes, which results in very high variability in relevés areas. The result is a biased representation of species richness, especially in species-poor plant communities where the sampler tends to enlarge the relevé size to include more species [53]. This influence on species count can indirectly affect the identification of the patterns of species occurrence at the community level. Thus, a standard plot size like the one used in this work seems much more suitable.

Other methodological issues in this study relate to the identification of generalist taxa and species of conservation value in our dataset. As regards generalist taxa, these were identified using a French study since it was the only one available. It should thus be taken into consideration that some species that behave as generalist in France could possibly behave differently in Italy. With regard to rare and threatened arable species, these were detected at the European level because single evaluations for each country have been made but not published in the used reference [34]. This implies that many arable plants that are considered rare or threatened at a continental level are actually very common in Italy. For instance, this is true in the case of *Ajuga chamaepitys, Filago pyramidata,* and *Lathyrus aphaca,* which are widespread even outside arable land in Italy [26]. Therefore, the development of a publicly-available list of Italian rare and threatened arable species is desirable in the near future, especially given the importance of Italy as a refuge for arable biodiversity [26,54].

Patterns of co-occurrence of rare or specialist taxa have also been observed in other kinds of plant communities, for instance, in trees in tropical and subtropical forests. In these cases, the evidence was explained by the low dispersal ability of the species [55,56]. To some extent, this is also true for many threatened arable species with heavy seeds such as *Agrostemma githago* and *Lolium temulentum*, which survive mainly by being reintroduced each year as impurities of crop seeds. Though there are various modes of seed dispersal, barochory is most frequent in typical arable weeds, which results in limited efficiency with regard to their spread outside fields [33].

Generalist species had a lower number of associations and most of these were negative associations with arable species of conservation value. This is consistent with the fact that generalist and very common taxa such as Chenopodium album or Sonchus oleraceus are able to colonize any kind of disturbed habitat. Such species also prefer soils with high nutrient amounts, contrary to typical winter arable weeds that thrive on nutrient-poor soils; this is consistent with their negative associations with the latter and their positive associations with each other. In a previous study in France, no positive associations of generalist arable species with other species were detected [6]. In this work, in which a co-occurrence analysis was conducted on the weeds of winter wheat in an intensive agricultural area, at the field and landscape levels, the lack of positive associations even between generalist species is probably due to the fact that it was purposely conducted in a very homogeneous environmental context. Here, the observed co-occurrence of specialist species of winter arable land at the field level was possibly related to favorable local management, i.e., low-intensity agricultural practices that allow typical species of cereal fields to thrive. Similarly, in our dataset, species of conservation value are clearly linked to extensively managed fields. This is especially the case for low-input fodder crops, like winter cereals (barley, oat) and cereal-legume mixtures, which are widespread in the mountains of central and southern Italy, where traditional agriculture is still common and very species-rich arable communities can be found (up to 40 species in 16 m²) [15]. The extensive management of such arable fields includes crop rotations, the absence of chemical weeding and the use of organic fertilizers (manuring). This contrasts with the intensive practices commonly used in industrial agricultural areas of northern Italy and the main plains of the Peninsula, which cause impoverishment and deterioration of arable biodiversity through extensive chemical weeding and fertilization, monocultures, and seed cleaning.

Besides the detected co-occurrence patterns, our analysis highlighted that 90% of the used species were randomly distributed in our plots. This evidence is also consistent with previous findings from western France, where the authors showed that arable species had a random distribution in more than 80% of the surveyed fields [6]. However, unlike the aforementioned study, our research was conducted in a very heterogenous environmental context. This may suggest that some of the detected positive associations could be due to species with similar ecological or management requirements aggregating in the same fields, i.e., they occupy similar niches.

Our results showed unexpected co-occurrence patterns for *Centaurea cyanus*, an obligate weed of winter cereals and similar crops. Previously, in two regions of France, the occurrence of *Centaurea cyanus* in winter arable fields was proved to be positively associated with that of other typical/rare arable taxa as *Adonis annua*, *Legousia speculum-veneris*, and *Ranunculus arvensis* [4,5]. In contrast with

this, our results showed that *C. cyanus* was one of the few species of conservation value that had no positive associations with other species of conservation value. Instead, it was positively associated to the generalist *Veronica arvensis* and *Viola arvensis*. This could be due to the fact that *C. cyanus* shares a preference for slightly acid soils with the latter two species (*Aperetalia spicae-venti* vegetation). As regards the mostly random distribution of *C. cyanus* across the study area, this could be due to the fact that this species, though under decline, is quite common in Italy and can even be found in several intensively managed agroecosystems.

5. Conclusions

The present study investigated, for the first time, the patterns of species co-occurrence in winter arable plant communities in mainland Italy, where arable plant biodiversity is particularly well-preserved, and which acts as a reservoir for arable species in Europe. The results suggest that rare and threatened arable plants have the potential to be indicators of areas with good preservation of typical arable plant communities, and of the overall good environmental quality of agroecosystems. The detected patterns of co-occurrence highlight that the occurrence of a valuable species can point to the presence of a valuable community, and that the presence of a generalist species can indicate the presence of a simplified community. The results suggest that further studies should be carried out to better clarify the usefulness of arable plant communities in agricultural and environmental management.

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