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Title Germination and emergence of annual species and burial depth: implications for restoration ecology.

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Abstract

Due to a high content of viable seeds, topsoil is usually spread on ground left bare during railway and motorway construction to facilitate the regeneration of vegetation cover. However, during handling of the topsoil, seeds are often buried deeply and they cannot germinate, or the seedlings cannot emerge from depth. This paper experimentally explores the predictive value of seed mass for seed germination, mortality and seedling emergence at different burial depths for 13 common annual species in semiarid Mediterranean environments. We separate the effect of burial depth on germination and emergence by means of two experiments. In the germination experiment, five replicates of 20 seeds for each species were buried at depths ranging from 0-4 cm under greenhouse conditions. Germinated and empty or rotten seeds were counted after 8 weeks. In the emergence experiment, five replicates of four newly-germinated seeds per species were buried at the same depths under controlled conditions and emergence was recorded after 3 weeks. The effect of burial depth on percentage of germination and seedling emergence was dependent on seed size.

Although all species showed a decrease in germination with burial depth, this decrease was greater for small- than large-seeded species. Percentage of emergence percentage was positively related to seed mass but negatively related to burial depth. Seed mortality was higher for small- than large-seeded species, but there was no general effect of burial depth on this variable. Thus, the current practice of spreading 30 cm deep layers of topsoil in post-construction restoration projects is inadvisable. In this restoration scenario thinner layers of topsoil should be used to achieve the maximum potential of the topsoil for germination and seedling establishment.

Key words: Mediterranean; Seedling; Seed burial experiment; Seed mortality; Suicide germination; Topsoil.

Nomenclature: Flora Europea (Tutin et al. 1964-1980)

Running head: Germination and emergence with burial depth

1. Introduction

In the ecological restoration of degraded zones such as embankments of linear infrastructure for roads and railways, a common practice in topsoil handling is to remove the upper 30-40 centimetres of soil prior to the start of the construction and later to spread it across areas devoid of vegetation in layers of the same depth (Newman and Redende 2001; Bote et al. 2005; Tormo et al. 2007; Mola et al. 2011; Rivera et al. 2012). This practice aims to facilitate the regeneration of plant cover due to the high content of viable native seeds, organic matter, nutrients and microorganisms in the

topsoil (Grant et al. 1996; Rokich et al. 2000; Holmes 2001; Moynahan et al. 2002; Tormo et al. 2007; García Palacios et al. 2011; Clewell and Aronson 2013; Rivera et al. 2014). However, the majority of the viable seeds are in the top 2-3 centimetres of topsoil (Fenner and Thompson 2005; Traba et al. 2006). Handling topsoil in this way dilutes the seed bank and often leaves the seeds at depths from where they cannot germinate, or the seedlings cannot emerge because they die before reaching the soil surface (fatal or suicide germination) (Rivera et al 2012).

Understanding the relationships between seed size, as an easy-to-measure plant trait, and seed germination and emergence with burial depth can help to facilitate topsoil handling strategies that are better suited to the functioning of soil seed banks in vegetation restoration projects. In the absence of dormancy, seedling emergence depends on two aspects related to burial depth: 1) seeds require favourable environmental conditions for germination including enough water, temperature, oxygen or light (Thompson and Ooi 2010), and 2) seedlings must grow to reach the soil surface to become established, and before the growth of a buried seedling consumes seed reserves (Fenner and Thompson 2005).

Many authors have studied the effect of burial beneath soil or litter on seedling germination or emergence in farm environments (Grundy and Mead 1998; Grundy et al. 1999; Kidson and Westoby 2000; Oliveira and Norsworthy 2006; Willson et al. 2006; Davis and Renner 2007), grasslands (Gulmon 1992; Traba et al. 2004; Bu et al 2007, Wu and Du 2007), woodlands (Peterson and Facelli 1992; Vasquez-Yanes and Orozco-Segovia 1992), sand dunes (Yanful and Maun 1996; Ren et al. 2002; Cordazzo et al 2002; Li et al. 2006) and deserts (Freas 1989; Pivatto et al. 2014). However, the majority of these studies have focused on one or very few species, and few multispecies

experiments, which are useful for detecting correlates with plant traits, have evaluated the predictive role of seed mass on germination at different depths (Pearson et al. 2002; Burmeier et al. 2010) and emergence of seedlings (Bond et al. 1999; Benvenuti 2001; Grundy et al. 2003; Burmeier et al. 2010). Furthermore, most studies that relate seed depth to emergence have focused on the seedlings that actually emerged rather than those that germinated but failed to reach the surface (Fenner and Thompson 2005).

Multispecies experiments have found that seed germination becomes less dependent on light as seed mass increases (Milberg et al. 2000), and small-seeded species have a higher survival rate during burial and are less likely to experience fatal germination than large-seeded species (Burmeier et al. 2010; Rivera et al. 2012). These results concur with the hypothesis proposed by various authors that the light response for germination (Milberg et al. 2000; Jankowska-Blaszczuk and Daws 2007) or the temperature fluctuation detection mechanism (Thompson et al. 1977; Benech-Arnold et al. 1988; Ghera et al. 1992; Liu et al. 2013; Koutsovoulou et al. 2014) can be adaptations to ensure that small-seeded species only germinate when close to the soil surface. Furthermore, the fact that species with small seeds tend to form more persistent seed banks than larger-seeded species (Thompson et al. 1993; Bekker et al. 1998; Peco et al. 2003) and small-seeded species are more likely to survive if they are buried more rapidly than large-seeded species (see e.g. Burmeier et al. 2010 for flooded systems; Rivera et al. 2012 for topsoil stockpiles).

Multispecies experiments also have found that large-seeded species seem to be more able to emerge when they are buried than small-seeded species. On farmland, for example, only the seeds near the soil surface were able to germinate and emerge

(Grundy et al. 2003). In the case of temperate herbaceous species on flood meadows, large-seeded species are able to emerge from greater depths and experienced less depth-mediated growth inhibition than small-seeded species (Burmeier et al. 2010). Both conditions are related to the greater potential for large seeds to develop large seedlings because they have greater reserves (see also Bond et al. 1999, who propose a predictive model of maximum depth for emergence based on seed mass).

Knowledge of the effect of seed mass in the response to artificial burial derived from topsoil handling during vegetation cover restoration is particularly relevant in semiarid Mediterranean environments because the vegetation is mainly composed of annual plants which regenerate from the seed bank each autumn. These seed banks contain a huge number of viable seeds (Levassor et al. 1990; Russi et al. 1992; more than 100,000 per m² in Ortega et al. 1997), although the majority of seeds for most species are in the top few centimetres (Traba et al. 2006).

With respect to burial depth, Traba et al. (2004) analyzed the emergence capacity of Mediterranean grassland seed banks artificially buried at different depths. They found that most species were unable to emerge when buried deeper than 1 cm, although their study did not determine the number of viable seeds available for germination. Experimental analysis in this type of environment also has investigated the effect of topsoil storage time on seed survival, germination and mortality (Rivera et al. 2012). It was found that after 6 months of topsoil storage, the viable seed bank was reduced by up to 60%, particularly in the case of large seeds (Rivera et al. 2012). One of the causes of this reduction was the germination of buried seeds of large-seeded species as a result

of their lack of burial detection mechanisms such as response to light or diurnal temperature fluctuation, which are common in small-seeded species.

In this study, we explore the importance of seed mass in the prediction of two processes related to seedling emergence after artificial burial: germination and the ability of the hypocotyl to reach the soil surface. Specifically, we aim to a) evaluate the response of seed germination and mortality to burial depth, and b) analyze the emergence ability of newly-germinated seedlings from different depths.

We conducted experiments under controlled conditions on 13 abundant annual species which form a gradient of seed mass. Our hypotheses were:

1) Germination of small-seeded species shows a greater dependence on burial depth than that of large-seeded species due to more effective sensing mechanisms aimed at avoiding fatal germination, with maximum germination near the soil surface.

2) Large-seeded species show less depth-induced seed mortality than small-seeded species due to the trade-off for a few large protected seeds vs. many small unprotected seeds.

3) Large-seeded species are expected to have higher seedling emergence when buried than small-seeded species due to their greater reserves.

2. Methods

The 13 species used in this study form a gradient in seed mass from 0.01 to 5.99 mg (Table 1), and their seed sizes covered approximately 90% of the range of herbaceous species growing in grasslands and old cereal croplands of central Spain (Sanchez et al. 2002, see Appendix A). For each species, seeds were collected in summer (July) from at least five populations in grasslands and old fields in the San Agustin de Guadalix municipality, 50 km north of Madrid (40°38'N, 3°70'E). Seeds were stored in paper bags in darkness at room temperature (approx. 20°C) for 4 months, until the start of the germination and seedling emergence experiments. Dry storage at room temperature allowed the seeds with physiological dormancy to after-ripen (Baskin and Baskin 2001). This is a common practice in germination experiments with seeds that require exposure to high summer temperatures before they can germinate annually in autumn (Baskin and Baskin 1982; Traba et al. 2004; Bretzel et al. 2009; Rivera et al. 2012).

2.2. Seed germination experiment

Five replicates of 20 clean, full intact seeds per species were placed in nylon bags, which were then placed in 20x30 cm trays containing a 5 cm vermiculite layer. The bags were covered with a 0, 1, 2, 3 or 4 cm layer of fine sand of 250 µm in diameter. The trays were left in a greenhouse for 8 weeks and watered every second day. The mean minimum and maximum temperatures recorded in the greenhouse during this period were -1.2 and 28.9°C. At the end of the experiment, we counted germinated, dead (empty or rotten) and intact non-germinated seeds, and for each replicate, we calculated percentage germination (number of germinated seeds / (number of germinated + intact seeds)) and the percentage mortality (number of dead seeds/20).

2.3. Seedling emergence experiment

In this experiment we used newly-germinated seedlings (hereinafter referred to as 'pre-germinated seedlings') to determine the effect of burial depth on the pre-emergence phase from the germination phase. Seeds were placed in Petri dishes on filter paper sitting on a vermiculite base, and left in a growth chamber with a light and temperature regime characteristic of September (22 °C/16 hours of light and 18 °C/8 hours of darkness) with a relative humidity of about 80%. Some seeds required pre-germination treatment (i.e. *Anthyllis lotoides*, *Brassica barrelieri*, *Rumex acetosella*, *Spergularia purpurea*, *Trifolium cherleri* and *Tuberaria guttata* were scarified, and those of *Andryala integrifolia* cold stratified). Scarification consisted of gently rubbing the seeds on a flat piece of fine grain sandpaper. Cold stratification involved leaving the seeds on a moist substrate at 5°C for 15 days until they were incubated at 22/18 °C. Low numbers of pre-germinated seedlings of *Thapsia villosa* excluded this species from the emergence experiment. Seedlings with 2 mm long radicles (ISTA, 2011) were transplanted to PVC tubes and buried at different depths. Seedlings that did not meet this requirement were rejected. For each species, five replicates of four seedlings were buried at depths of 1, 2, 3 and 4 cm in the chosen substrate. Prior to transplanting seedlings, the silty substrate from the study area was sterilized and packed into PVC tubes (5 cm diameter x 20 cm length).. The PVC tubes were put in a Phytotron for 3 weeks with a daily regime of 16h of light at 22° C and 8 h of darkness at 16° C. The samples were kept moist throughout the period. At the end of the experiment, the number of emerged seedlings were recorded and used to calculate percentage

emergence (number of emerged seedlings / number of pre-germinated seedlings). At the end of the experiment, the tubes were checked for live seedlings that had not emerged.

2.4. Statistical analysis

Binomial models with logit link (Crawley et al. 2005) were used to analyse the relationship between seed germination, seed mortality and seedling emergence percentages as response variables, and seed mass and burial depth as explanatory variables. Seed mass was log-transformed. When significant interactions between seed mass and depth were detected, the analysis of the effect of depth on the response variables was done for small and large seeds separately (seed mass <0.28 mg or ≥ 0.28 mg, respectively, see Appendix A, for median seed mass distribution of the herbaceous species in the study area, Fig A.1). In these cases, we used mixed binomial models with “species” as a random factor. All the statistical analyses were done with R.3.1.2 (R Development Core Team 2014). R software package lme4 was used for Mixed Effects models (Bates et al., 2014).

3. Results

The percentages of seed germination, seed mortality and seedling emergence were highly variable between species, ranging from 1.2 to 98% for seed germination, from 0 to 47 % for seed mortality and 0 to 97% for seedling emergence (Table1). The effect of burial depth on seed germination and seed mortality depended on seed size (see Table 2 a, d for significant interactions). Although all species showed a decrease in germination with burial depth, this decrease was higher in the case of small- than large-seeded

species (regression coefficients of -0.64 and -0.19, respectively, Table 2 b, c and Fig. 1). Seed mortality was in general very low, but it was lower for large- than for small-seeded species (18 ± 19 and 7 ± 13 for small- and large-seeded species, respectively, Fig 2). Seed mortality was not dependent on burial depth for either species groups (Table 2 e, f). Percentage of emergence of pre-germinated seedlings was very low in small-seeded species compared to large-seeded ones (3 ± 16 and 60 ± 39 , respectively), and it tended to decline with depth in both cases (Table 2 g, Fig 3).

4. Discussion

4.1. Effects of burial depth on seed germination and mortality

We found an overall decrease in the percentage germination with increasing burial depth, but this decrease was higher for small- than large-seeded species, which supports our first hypothesis. This pattern could be explained by the positive relationship that has been found between seed size and seedling size (Leishman et al. 2000, Lahoreau et al. 2006), which is responsible for small seedlings being less likely to survive in competition with established plants and to emerge from deeper soil than large seedlings (Pearson et al. 2002). It is thus reasonable to assume that small-seeded species are subject to selection pressure to develop gap-detection mechanisms such as light sensitivity, which can be used by the seed to distinguish between “soil surface” or “buried” (Woolley and Stoller 1978, Benvenuti 1995), or temperature fluctuation sensitivity, which provides further information about the burial depth (Thompson and Grime 1983). For example, germination experiments in the dark and light have shown that germination becomes less dependent on light with increasing seed mass, even when

considering phylogenetic independent contrasts, which suggest that light response and seed mass coevolved (Milberg et al. 2000).. Furthermore, although the seed mass range in this study was larger than the one used in our germination experiment, 90% of their species are included in our range of seed mass. Liu et al. (2013) found that small-seeded species (less than 1 mg) were more likely than large-seeded species to respond positively to fluctuating temperatures in germination experiments. Our results also concur with those of Rivera et al. (2012) for 10 annual Mediterranean species with seed mass ranging from 0.02 to 1.68 mg and buried at different depths in topsoil stockpiled for 6 months. These authors also found a negative relationship between percentage germination and the presence of light or diurnal temperature fluctuation responses, as gap-detection mechanisms in small-seeded species.

Our second hypothesis also was supported by the results of the germination experiment, as seed mortality was lower in large-seeded than small-seeded-species. However, seed mortality was generally very low. Lower seed mortality for large-seeded species than for small ones was also found by Rivera et al. (2012) in topsoil stockpiles. Several reasons for this result have been suggested, such as the higher investment of large-seeded species in their seed coats, which protect them from pathogen attack (Crist and Frieser 1993; Baskin and Baskin 2001) or from olfactive detection by granivores (Paulsen et al. 2014). However, contrary to Rivera et al. (2012), we only found a marginal effect of burial depth on seed mortality for large-seeded species, probably due to the longer burial periods and higher burial depths explored in their experiment.

4.2. Effects of burial depth on seeding emergence

Our third hypothesis also was supported. The emergence of pre-germinated seedlings was very low in small-seeded species compared to large-seeded, and it tended to decline with depth. Furthermore, almost none of the small-seeded species were able to emerge if buried below 2cm of soil, as shown in a previous study (Traba et al. 2004). One might argue that the result of this emergence experiment could be explained by a lower relative growth rate of seedlings from small-seeded species in relation to large ones, but our own observations and evidence in the literature permit this argument to be disregarded for the following reasons. In this experiment, we did not measure the relative growth rate of pre-germinated seedlings, but we did check the number of live seedlings that had not emerged at the end of the three week experiment. This number was lower than or equal to 2 seedlings for the majority of species, except for the heavier seeded species, *Anthyllis lotoides* (14 seedlings) and *Trifolium cherleri* (5 seedlings). We may thus disregard the possibility that small-seeded species did not have time to emerge from depth in the course of the experiment. Furthermore, other studies suggest that there is a higher relative growth rate of seedlings from small-seeded species than large ones (Leishman et al. 2000). Small-seeded species produce seedlings that are more susceptible to physical damage, which can contribute to the high mortality, even when pre-germinated seedlings are buried at shallow depths (Grundy et al. 2003)

Our results are in line with other experiments using pre-germinated seedlings that permit the separation of the effect of burial depth on the pre-emergence phase from the germination phase (Bond et al. 1999, Grundy et al. 2003). Although seedling emergence for large-seeded species was higher than for small-seeded species in our experiment, large-seeded species only comprise a small fraction of the seed banks in

annual Mediterranean grasslands due to the general trade-offs between seed mass and seed number (Venable 1992). and the greater ability of small-seeded species to form persistent seed banks, even for seed masses similar to those in our experiment (Peco et al. 2003). Furthermore, large-seeded herbaceous species would probably be unable to emerge after germinating at deeper layers than those tested in our experiment, considering that the emergence percentage decreases with a regression coefficient of -0.27. In other words, on the basis of our experiment, we can predict emergence percentages close to 0 at depths of more than 10cm. This can have important implications for restoration ecology. Similar declines in seedling emergence of herbaceous species with burial depth have been found in weed species with seed masses ranging from 11.41 to 0.08 g (Benvenuti et al. 2001). These authors found that weed emergence in arable systems only occurs in the upper 10 cm of soil, with clear implications for weed management.

5. Conclusions and implications for restoration projects

Seed mass can be used as a predictor of seed germination and seedling emergence in relation to burial depth in Mediterranean annual species. Our results show that small-seeded annual species, the majority component of the seed banks, do not germinate or emerge when buried deeper than 2cm. Therefore they cannot contribute to the vegetation recovery when deep layers of topsoil are spread on bare ground. Even in large-seeded species, the germination percentage declines with burial depth, and, although their seedling emergence is higher than small-seeded species, large-seeded

herbaceous species probably cannot emerge when germinating at depths greater than 10cm.

The results of this experiment suggest that the current practice of spreading 30 cm deep layers of topsoil in restoration projects connected with construction of roads and railways is inappropriate. Thinner layers of topsoil should be used to promote the maximum potential of the topsoil for germination and seedling establishment. Furthermore, a viable seed content very small below the first 3-5cm of topsoil (Fenner and Thompson 2005; Traba et al. 2006) also suggests that a thin layer could be used instead of the usual 30-40 cm of topsoil spread for restoration purposes. In an experimental study of topsoil handling for restoration of *Banksia* woodlands in Australia, Rokich et al. (2000) also recommended the spread of topsoil to a maximum depth of 10cm in order to optimize revegetation. Nevertheless, the use of deep layers of topsoil also can be recommended if the restoration is aimed at improving the soil texture, penetrability or nutrients, although in this case, the shallow and deep topsoil layers should be managed independently and the vertical layer's structure should be regenerated (Bulot et al 2014).

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Author contributions:

BP conceived and designed the research; AL did the experiments; BP and AL analysed the data; BP contributed reagents/materials/analysis tools; BP and AL wrote and edited the manuscript

References

- Baskin, J.M., Baskin, C.C., 1982. Germination ecophysiology of *Arenaria glabra*, a winter annual of sandstone and granite out-crops of south-eastern United States. *Am. J. Bot.* 69, 973-987.
- Baskin, C.C., Baskin, J.M., 2001. Seeds: Ecology, Biogeography and Evolution of Dormancy and germination. Academic Press, San Diego, CA, US.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear Mixed-effects Models Using Eigen and S4. R package version 1.0-5. <http://CRAN.R-project.org/package=lme4>
- Bekker, R.M., Bakker, J.P., Grading, U., Kalamees, R., Milberg, P., Poschlod, P., Thompson, K., Willems, J.H., 1998. Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Funct. Ecol.* 12, 834-842.
- Benech-Arnold, R.L., Ghersa, C.M., Sánchez, R.A., García-Fernández, A.E., 1988. The role of fluctuating temperatures in the germination and establishment of

371 *Sorghum halepense* (L.) Pers. Regulation of germination under leaf canopies.
372 Funct. Ecol. 2, 311–318.

373 Benvenuti, S., 1995. Soil light penetration and dormancy of Jimsonweed (*Datura*
374 *stramonium*) seeds. Weed Sci. 43, 389-393.

375 Benvenuti, S., Macchia, M., Miele, S., 2001. Quantitative analysis of emergence of
376 seedlings from buried weed seeds with increasing soil depth. Weed Sci. 49 (4),
377 528-535.

378 Bond, W.J., Honig, M., Maze, K.E., 1999. Seed size and seedling emergence: an
379 allometric relationship and some ecological implications. Oecologia 120, 132–
380 136.

381 Bote, D., Valladares, F.J., Matesanz, S., Tena, D., 2005. Importancia de la tierra vegetal
382 en la vegetación de desmonte. Ciencia y Técnica 80, 19-24.

383 Bretzel, F., Pezzarossa, B., Benvenuti, S., 2009. Soil influence on the performance of 26
384 native herbaceous plants suitable for sustainable Mediterranean landscaping.
385 Acta Oecol. 35, 657-663.

386 Bu, H.Y., Chen, X.L., Xu, X.L., Liu, K., Jia, P., Du, G.Z., 2007. Seed mass and
387 germination in an alpine meadow on the eastern Tsinghai-Tibet plateau. Plant
388 Ecol. 191(1), 127-149.

389 Bulot, A., Provost, E. Dutoit, T., 2014. Comparison of different soil transfer strategies
390 for restoring a Mediterranean steppe after a pipeline leak (La Crau plain, South-
391 Eastern France). Ecol. Eng. 71, 690-702.

392 Burmeier, S., Donath, T.W., Otte, A., Eckstein, R.L., 2010. Rapid burial has differential
393 effects on germination and emergence of small- and large-seeded herbaceous
394 plant species. Seed Sci. Res. 20(3), 189-200.

395 Clewell, A.F., Aronson, J., 2013. Ecological restoration: Principles, Values and
 396 Structure of an Emerging Profession, second ed. Island Press, Washington,
 397 US. Cordazzo, C.V., 2002. Effect of seed mass on germination and growth in
 398 three dominant species in southern Brazilian coastal dunes. *Braz. J. Biol.* 62 (3),
 399 427-435.

400 Crist, T.O., Frieser, C.F., 1993. The impact of fungi on soil seeds: implications for
 401 plants and granivores in a semiarid shrub-steppe. *Ecology* 74, 2231-2239.

402 Crawley, M.J., 2005. Statistics: An Introduction using R. Wiley & Sons Chichester.
 403 UK.

404 Davis, A.S., Renner, K.A., 2007. Influence of seed depth and pathogens on fatal
 405 germination of velvetleaf (*Abutilon theophrasti*) and giant foxtail (*Setaria*
 406 *fabry*). *Weed Sci.* 55, 30-35.

407 Fenner, M., Thompson, K., 2005. The ecology of seeds. Cambridge University Press,
 408 Cambridge, UK.

409 Freas, P.R., 1989. Seed banks and vegetation processes in deserts, in: Leck, M.A.,
 410 Parker, V.T., Simpson, R.L. (Eds.), Ecology of soil seed banks. Academic Press,
 411 San Diego, US, pp. 257–281.

412 Garcia-Palacios, P., Bowker, M.A., Chapman, S.J., Maestre, F.T., Soliveres, S.,
 413 Gallardo, A., Valladares, F., Guerrero, C., Escudero, A., 2011. Early-
 414 successional vegetation changes after roadside prairie restoration modify
 415 processes related with soil functioning by changing microbial functional
 416 diversity. *Soil Biol. Biochem.* 43(6), 1245-1253.

417 Ghera, C.M., Benech-Arnold, R.L., Martínez-Ghera, M.A., 1992. The role of
 418 fluctuating temperatures in germination and establishment of *Sorghum*

419 *halepense*. Regulation of germination at increasing depths. *Funct. Ecol.* 6, 460–
420 468.

421 Grant, C.D., Bell, D.T., Koch, J.M., Loneragan, W.A., 1996. Implications of Seedling
422 Emergence to Site Restoration following Bauxite Mining in Western Australia.
423 *Restor. Ecol.* 4(2), 146-154.

424 Grundy, A.C., Mead, A., 1998. Modelling the effects of seed depth on weed seedling
425 emergence. *Asp. Appl. Biol.* 51, 75–82.

426 Grundy, A.C., Mead, A., Burston, S., 1999. Modelling the effect of cultivation on seed
427 movement with application to the prediction of weed seedling emergence.
428 *J. Appl. Ecol.* 36, 663–678.

429 Grundy, A.C., Mead, A., Burston, S., 2003. Modelling the emergence response of weed
430 seeds to burial depth: interactions with seed density, weight and shape. *J. Appl.*
431 *Ecol.* 40, 757–770.

432 Gulmon, S.L., 1992. Patterns of seed germination in Californian serpentine grassland.
433 *Oecologia* 89, 27-31.

434 ISTA, 2011. International Rules for Seed Testing. International Seed Testing
435 Association, Bassersdorf

436 Holmes, P.M., 2001. Invasion and mining: Effects of topsoil depth, seed source, and
437 fertilizer addition. *Restor. Ecol.* 9, 71-84.

438 Jankowska-Blaszczuk, M., Daws, M.I., 2007. Impact of red:far red ratios on
439 germination of temperate forested herbs in relation to shade tolerance, seed mass
440 and persistence in the soil. *Funct. Ecol.* 21, 1055-1062.

441 Kidson, R., Westoby, M., 2000. Seed mass and seedling dimensions in relation to
442 seedling establishment. *Oecologia* 125, 11-17.

443 Koutsovoulou, K., Daws, M.I., Thanos, C.A., 2014. *Campanulaceae*: a family with
 444 small seeds that require light for germination. *Ann. Bot.* 113, 135-143.

445 Lahoreau, G., Barot, S., Gignoux, J., Hoffmann, W.A., Setterfield, S.A., Williams, P.R.,
 446 2006. Positive effect of seed size on seedling survival in fire-prone savannas of
 447 Australia, Brazil and West Africa. *J.Trop.Ecol.* 22, 719–722.

448 Leishman, M.R., Wright, I.J., Moles, A.T., Westoby, M., 2000. The Evolutionary
 449 Ecology of seed size, in: Fenner, M. (ed.), *Seeds- The Ecology of regeneration*
 450 *of Plant Communities*, CAB International, Wallingford, UK, pp. 31-57.

451 Levassor, C., Ortega, M., Peco, B., 1990. Seed banks dynamics of Mediterranean
 452 pastures subjected to mechanical disturbance. *J.Veg.Sci.* 1, 339–344.

453 Li, Q.Y., Zhao, W.Z., Fang, H.Y., 2006. Effects of sand burial depth and seed mass on
 454 seedling emergence and growth of *Nitraria sphaerocarpa*. *Plant Ecol.* 185, 191-
 455 198.

456 Liu, K., Baskin, J.M., Baskin, C., Bu, H.Y., Du, G.Z., Ma, M.J., 2013. Effect of Diurnal
 457 Fluctuating versus Constant Temperatures on Germination of 445 Species from
 458 the Eastern Tibet Plateau . *PLOS ONE* 8 (7), e69364.

459 Milberg, P., Andersson, L., Thompson, K., 2000. Large-seeded species are less
 460 dependent on light for germination than small-seeded ones. *Seed Sci. Res.* 10,
 461 99-104.

462 Mola, I., Jiménez, M.D., López-Jiménez, N., Casado, M.A., Balaguer, L., 2011.
 463 Roadside reclamation outside the revegetation season: Management options
 464 under schedule pressure. *Restor. Ecol.* 19, 83-92.

465 Moynahan, O.S., Zabinski C.A., Gannon, J.E., 2002. Microbial community structure
 466 and carbon-utilization diversity in a mine tailings revegetation study. *Restor.*
 467 *Ecol.* 10(1), 77-87.

468 Newman, G.J., Redente, E.F., 2001. Long-term plant community development as
 469 influenced by revegetation techniques. *J. Range Manage.* 54, 717-724.
 470
 471 Oliveira, M.J., Norsworthy, J.K., 2006. Pitted morning glory (*Ipomea lacunosa*)
 472 germination and emergence as affected by environmental factors and seedling
 473 depth. *Weed Sci.* 54, 910-916.
 474 Ortega, M., Levassor, C., Peco, B., 1997. Seasonal dynamics of Mediterranean pasture
 475 seed banks along environmental gradients. *J. Biog.* 24, 177–195.
 476 Paulsen, T.R., Hogstedt, G., Thompson, K., Vandvik, V., Eliassen, S., 2014. Conditions
 477 favouring hard seededness as a dispersal and predator escape strategy. *J. Ecol.*
 478 102(6), 1475-1484.
 479 Pearson, T.R.H., Burslem, D.F.R.P., Mullins, C.E., Dalling, J.W., 2002. Germination
 480 ecology of neotropical pioneers: interacting effects of environmental conditions
 481 and seed size. *Ecology* 83, 2798–2807.
 482 Peco, B., Traba, J., Levassor, C., Sánchez, A., Azcárate, F.M., 2003. Seed size, shape
 483 and persistence in dry Mediterranean grass and scrubland. *Seed Sci. Res.* 13, 87–
 484 95.
 485 Peterson, C.J., Facelli, J.M., 1992. Contrasting germination and seedling growth of
 486 *Betula allegheniensis* and *Rhus typhina* subjected to various amounts and types
 487 of plant litter. *Am. J. Bot.* 79, 1209-1216.
 488 Pivatto, M.S., Funes, G., Ferreras, A.E., Gurvich, D.E., 2014. Seed mass, germination
 489 and seedling traits for some central Argentinian cacti. *Seed Sci. Res.* 24, 71-77
 490 R Development Core Team. 2014. R a language and environment for statistical
 491 computing. Viena , R foundation for Statistical Computing

492 Ren, J., Tao, L., Liu, X.M., 2002. Effect of sand burial depth on seed germination and
 493 seedling emergence of *Calligonum* L. species. J. Arid Environ. 51, 603-611.
 494 Rivera, D., Jáuregui, B.M., Peco, B., 2012. The fate of herbaceous seeds during topsoil
 495 stockpiling: restoration potential of seed banks. Ecol. Eng. 44, 94-101.
 496 Rivera, D., Mejias, V., Jauregui, B.M., Costa-Tenorio, M., Lopez-Archilla, A.I., Peco,
 497 B., 2014. Spreading Topsoil Encourages Ecological Restoration on
 498 Embankments: Soil Fertility, Microbial Activity and Vegetation Cover. PLOS
 499 ONE 9(7), e101413
 500 Rokich, D.P., Dixon, K.W., Sivasithamparam, K., Meney, K.A., 2000. Topsoil handling
 501 and storage effects on woodland restoration in western Australia. Restor. Ecol. 8,
 502 196-208.
 503 Russi, L., Cocks, P.S., Roberts, E.H., 1992. Seed bank dynamics in a Mediterranean
 504 grassland. J. Appl. Ecol. 29, 763–771.
 505 Sánchez, A.M., Azcárate, F.M., Arqueros, L., Peco, B., 2002. Volumen y dimensiones
 506 como predictors del peso de semilla en especies herbáceas del centro de la
 507 Península Ibérica. Anales Jard. Bot. Madrid 59, 249-262.
 508 Thompson, K., Grime, J.P., Mason, G., 1977. Seed germination in response to diurnal
 509 fluctuations in temperature. Nature 267, 147-148
 510 Thompson, K., Grime, J.P., 1983. A comparative study of germination responses to
 511 diurnally-fluctuating temperatures. J. Appl. Ecol. 20, 141-156.
 512 Thompson, K., Band, S.R., Hodgson, J.G., 1993. Seed size and shape predict
 513 persistence in soil. Funct. Ecol. 7, 236-241.
 514 Thompson, K., Ooi, M.K.J., 2013. Germination and dormancy breaking: two different
 515 things. Seed Sci. Res. 23(1), 1-1.

516 Tormo, J., Bochet, E., García-Fayos, P., 2007. Roadfill revegetation in semiarid
 517 Mediterranean environments. Part II: Topsoiling, species selection, and
 518 hydroseeding. *Restor. Ecol.* 15, 97-102.

519 Traba, J., Azcárate, F.M., Peco, B., 2004. From what depth do seeds emerge? A soil
 520 seed bank experiment with Mediterranean grassland species. *Seed Sci. Res.* 14,
 521 297–303.

522 Traba, J., Azcárate, F.M., Peco, B., 2006. The fate of seeds in Mediterranean soil seed
 523 banks in relation to their traits. *J.Veg.Sci.* 17, 5-10.

524 Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters,
 525 D.H., Webb, D.A., 1964-1980. *Flora Europea*. Cambridge University Press.
 526 Cambridge, UK

527 Vasquez-Yanes, S.C., Orozco-Segovia, A., 1992. Effects of litter from tropical
 528 rainforest on tree seed germination and establishment under controlled
 529 conditions. *Tree Physiol.* 11, 391-400.

530 Venable, D.L., 1992. Size-number trade-offs and the variation of seed size with plant
 531 resource status. *Am. Nat.* 140(2), 287-304.

532 Wilson, D.G., Burton, M.G., Spears, J.E., York, A.C., 2006. Doveweed (*Murdannia*
 533 *nudiflora*) germination and emergence as affected by temperature and seed
 534 burial depth. *Weed Sci.* 54, 1000-1003.

535 Woolley, J.T., Stoller, E.W., 1978. Light penetration and light-induced seed
 536 germination in soil. *Plant Physiol.* 61, 597-600.

537 Wu, G., Du, G., 2007. Germination is related to seed mass in grasses (*Poaceae*) of the
 538 eastern Qinghai-Tibetan Plateau, China. *Nord. J. Bot.* 25(5-6), 331-365.

539 Yanful, M., Maun, M.A., 1996. Effects of burial of seeds and seedlings from different
540 seed sires on the emergence and growth of *Strophostyles helvola*. Can. J. Bot.
541 74, 1322-1330.
542

543 Table 1 Seed mass and seed germination, seed mortality and seedling emergence percentages (mean \pm sd) of the species used in the
544 experiments. The percentages were calculated for each replicate and burial depth treatment before the calculation of the mean and sd for all
545 the treatments The total number of live seedlings that did not emerge are also included. ND: no data.

Species	Family	Seed mass (mg)	Germination (%)	Mortality (%)	Emergence (%)	Live seedlings number
<i>Andryala integrifolia</i>	Asteraceae	0.19	59.2 \pm 21.7	47.6 \pm 15.7	0.0 \pm 0.0	0
<i>Anthemis arvensis</i>	Asteraceae	0.72	38.3 \pm 35.4	19.8 \pm 13.7	16.3 \pm 23.3	2
<i>Anthyllis lotoides</i>	Leguminosae	1.23	4.6 \pm 4.6	1.0 \pm 2.5	75.0 \pm 35.4	14
<i>Brassica barrelieri</i>	Brassicaceae	0.74	9.6 \pm 9.1	0.2 \pm 1.0	73.3 \pm 30.6	0
<i>Bromus hordeaceus</i>	Poaceae	0.61	97.8 \pm 5.6	0.0 \pm 0.0	97.2 \pm 8.1	0
<i>Filago lutescens</i>	Asteraceae	0.02	33.1 \pm 34.1	7.8 \pm 8.8	5.0 \pm 19.4	0
<i>Plantago coronopus</i>	Plantaginaceae	0.16	80.3 \pm 26.9	2.0 \pm 3.2	1.3 \pm 5.6	0
<i>Rumex acetosella</i>	Polygonaceae	0.36	9.5 \pm 11.7	4.6 \pm 6.1	16.3 \pm 36.5	1
<i>Spergularia purpurea</i>	Caryophyllaceae	0.02	59.8 \pm 33.6	32.0 \pm 21.5	0.0 \pm 0.0	1
<i>Tapsia villosa</i>	Umbelliferae	5.99	67.2 \pm 28.7	23.2 \pm 16.5	ND	ND
<i>Tolpis barbata</i>	Asteraceae	0.10	33.6 \pm 34.4	16.4 \pm 12.3	2.5 \pm 7.7	2
<i>Trifolium cherleri</i>	Leguminosae	1.55	1.2 \pm 2.2	0.4 \pm 1.38	59.8 \pm 34.2	5
<i>Tuberaria guttata</i>	Cistaceae	0.04	5.4 \pm 7.6	17.0 \pm 9.7	0.0 \pm 0.0	1

546 Table 2. Parameter estimates and significance levels for the fitted binomial models for
 547 seed germination, seed mortality and seedling emergence percentages. Small seeds: seed
 548 mass<0.28 mg, large seeds: seed mass \geq 0.28mg. The seed mass cut-off point is the
 549 median of the seed mass distribution of Fig A.1.
 550

	B	SE(B)	z-value	P
<i>a) Germination</i>				
Log seed mass	-2.64	0.29	-9.22	<0.001
0.127.67 <0.001Burial depth	-0.59	0.03	-18.74	<0.001
<i>b) Germination (only small seeds)</i>				
Seed mass x Burial depth				
Burial depth	-1.18	0.05	-21.64	<0.001
<i>c) Germination (only large seeds)</i>				
Burial depth	-0.45	0.05	-9.45	<0.001
<i>d) Mortality</i>				
Log seed mass	-3.15	0.50	-6.28	<0.001
Burial depth	-0.04	0.04	-1.18	0.237
Seed mass x Burial depth	0.51	0.19	2.77	<0.01

e) Mortality (only small seeds)

Burial depth	0.01	0.03	0.07	0.944
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f) Mortality (only large seeds)

Burial depth	0.10	0.06	1.89	0.058
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g) Emergence

Log seed mass	13.19	2.50	5.28	<0.001
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Burial depth	-0.37	0.20	-1.87	0.062
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Seed mass x Burial depth	0.16	0.96	0.17	0.864
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551

Figure captions

Figure 1. Relationship between seed germination and burial depth. Species classified by seed mass (circles: small seeds, seed mass < 0.28 mg; triangles: large seeds, seed mass ≥ 0.28 mg). The seed mass cut-off point is the median of the seed mass distribution shown in Fig A.1.

Figure 2. Relationship between seed mortality and burial depth. Species classified by seed mass (circles: small seeds, seed mass < 0.28 mg; triangles: large seeds, seed mass ≥ 0.28 mg). The seed mass cut-off point is the median of the seed mass distribution shown in Fig A.1.

Figure 3. Relationship between seedling emergence and burial depth. Species classified by seed mass (circles: small seeds, seed mass < 0.28 mg; triangles: large seeds, seed mass ≥ 0.28 mg). The seed mass cut-off point is the median of the seed mass distribution of Fig A.1.

Figure A.1. Histogram of seed mass for the 118 most abundant herbaceous species in the northern part of the Madrid region (data taken from Sanchez et al. 2002)