

## Supplementary file S1: Additional information on methods

### Text A.1. Selection of transects and sampling points

In the study area in Södermanland county in Central Sweden is a sampling grid of 50 square transects (1x1km) established. These transects are several kilometers apart from each other. On these transects dung counts have been performed just after snowmelt (March-April) yearly from 2012 onwards, to establish the presence and abundance of five ungulate species: fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), moose (*Alces alces*) and wild boar (*Sus scrofa*). Pellet counts have been performed on 16 sampling points on each transect (four on each side of the square) as described in Spitzer et al. (2021). Based on these pellet counts from 2016, 2017 and 2018 we selected 20 transects comprising a gradient of densities of the different ungulate species. Per transect the pellet counts have been performed at sixteen sampling points, and therefore for each transect the average pellet count per species per year was calculated using equation [A.1]:

$$\mu_{t_{iy}} = \frac{\sum_{p=1}^{16} d_{p_{ty}}}{16} \quad [\text{A.1}]$$

where  $\mu_{t_{iy}}$  is the average pellet count on transect  $t$  for species  $i$  in year  $y$  and  $d_{p_{ty}}$  is the pellet count on sampling point  $p$  on transect  $t$  for species  $i$  in year  $y$ . Per species per year the transects have been ranked from high to low, so the transect with the highest average pellet count in a year got ranked as 1 and the transect with the lowest average pellet count got ranked as 50. The average rank of the transect per species of the years 2016, 2017 and 2018 was calculated using the equation [A.2]:

$$r_{it} = \frac{\sum_{y=2016}^{2018} k_{it_y}}{3} \quad [\text{A.2}]$$

where  $r_{it}$  is the average ranking of species  $i$  on transect  $t$  and  $k_{it_y}$  is the ranking of species  $i$  on transect  $t$  in year  $y$ . If the average ranking of a species on a transect was 25 or lower, the species was considered to be present in high densities on that transect. If the average ranking of a species on a transect was higher than 25, the species was considered to be present in low densities on that transect. Based on the average ranking of the five ungulate species, we selected 20 transects with as much as possible a gradient among the five ungulate species with regards to low and high densities, and with at least 8 sampling points located in forest according to Google Earth pro. Permission was obtained from landowners on 18 of the 20 selected transects. The other two selected transects were replaced with other transects, based on the fact that we had permission from landowners on those transects and that the gradient of ranking of the different ungulate species was fairly similar to the transects that needed to be replaced.

On each side of the transect four sampling points are present, which are situated 200m apart. So in total there are 16 sampling points, which are numbered clockwise (Figure A.1). On the selected transects we selected eight sampling points, based on two selection requirements: 1) the sampling point had to be situated in the forest according to Google Earth pro and 2) the sampling points needed to be as spread out over the transect as possible. Based on a decision tree we selected eight sampling points per transect (Figure A.2).

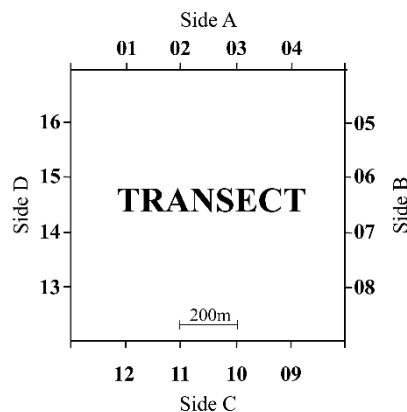


Figure A.1. Schematic layout of the square transects with the sampling points.

Due to circumstances in the field, some of the selected sampling points were not suitable for the collection of tick and vegetation data. For example, when a point was situated in a clear cut (Figure A.3A and C) or when the ground was covered with too much logs and branches (Figure A.3B). A clear cut was defined as a part of a forest where minimal 70% of the trees were 5m or lower. Sampling points which were not suitable for the collection of tick- and vegetation data were replaced by another sampling point based on a decision tree (Figure A.4). For nine transects it was not possible to select another sampling point, and thus less than eight sampling points were sampled on those transects (six with seven points and three with six points). Sampling points that were replaced were only replaced for the collection of tick- and vegetation data, not for the collection of ungulate data due to logistics with landowner permission.

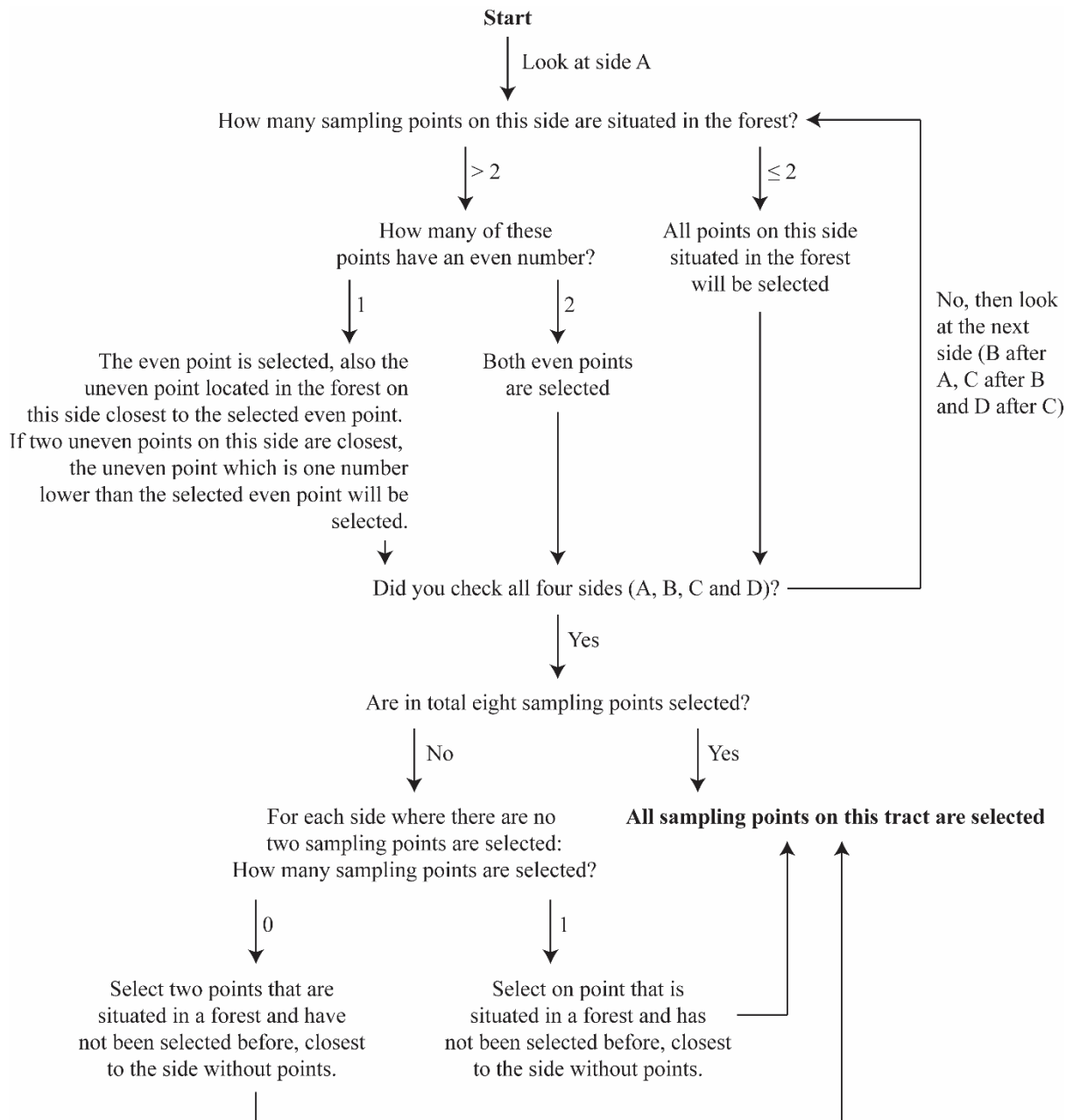


Figure A.2. Decision tree for the sampling point selection.

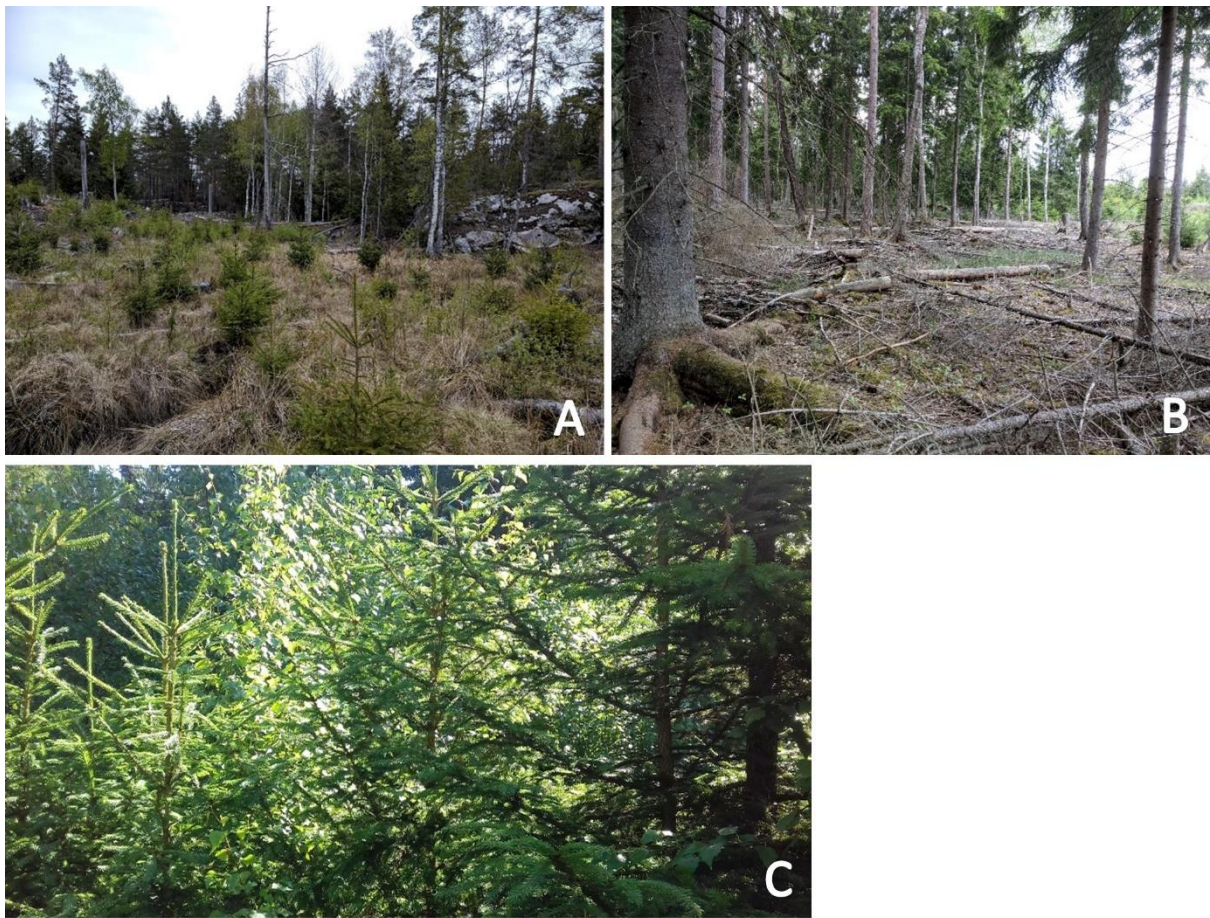


Figure A.3. Examples of sampling points that were excluded during the in field-selection. A) A sampling point which was in a younger clear cut (minimal 70% trees lower than 5m). B) A sampling point where too much logs and branches were present. C) A sampling point which was in an older clear cut, but still there were less than 30% trees above 5m.

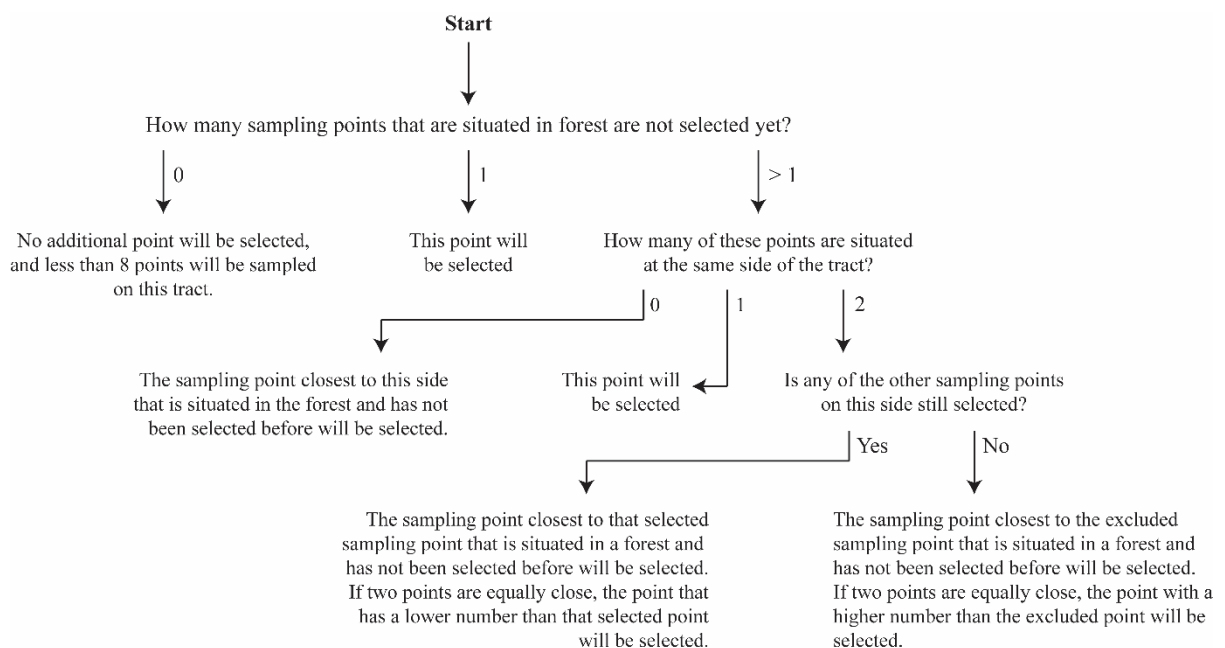


Figure A.4. Decision tree for the selection of replacing sampling points when sampling points that were selected with the decision tree in Figure A2 had to be excluded based on observations in the field.

### Text A2. Tick and field layer height collection

On each sampling point we collected ticks by dragging a 1m<sup>2</sup> white cloth over the vegetation. A total of 100m was dragged, divided into transects of 10m. Around each sampling point we estimated a circle with a radius of 30m. Within this circle we laid down the transect with the aid of a 10m rope, on locations that were most optimal for dragging (Figure A.5). Eight transects were laid down on lines in each wind direction: North, South, East, West, Northeast, Northwest, Southeast and Southwest. The other two transects were laid down perpendicular on North and South, without crossing any of the other transects (Figure A.6). The dragging was performed with a speed of approximately 3 km/h. After dragging 10m, the white cloth was turned over and the number of ticks on the cloth were counted. All stadia of ticks were counted separately: larvae, nymphs, female adults and male adults. All nymphs and adults have been collected individually in 8-strip Eppendorf tubes, also nymphs and adults found crawling on the field staff. All collected ticks were stored at -18°C at the end of each collection day, awaiting further analyses.



Figure A.5. Two examples of the 10m transects, laid out with the aid of a rope, where the tick collection took place.

For each 10m transect the temperature at approximately 1m height was noted. Furthermore we collected the height of the field layer on 5 points of the 10m transect, with 2.5m between them (Figure A.6). We determined the field layer as vegetation with a maximum height of 50cm. The height of the field layer was determined using the drop disc method, where a lightweight disc with a radius of 30cm with a central slot was dropped down a vertically held measuring stick, and the height above ground where it comes to rest is measured. If the disc hits vegetation that does not classify as field layer, the measuring point was moved along the dragging line until the disc only hits field layer vegetation.

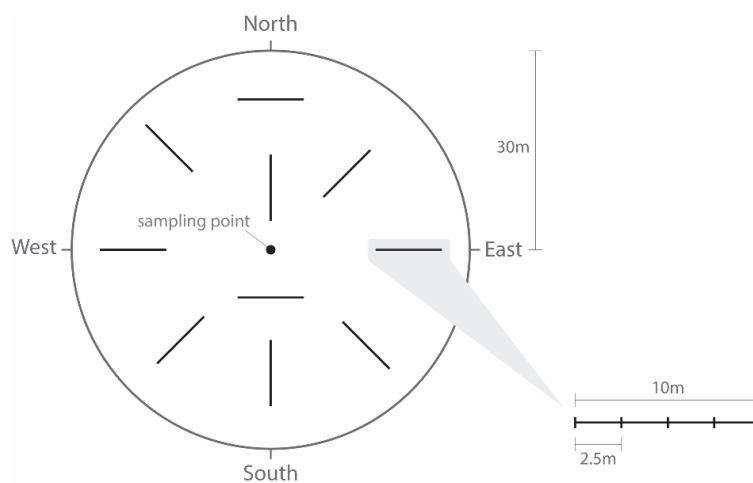


Figure A6. Schematic representation of an example of the placement of the 10m transects. The 10m transects were laid down anywhere on a line in each of the eight wind directions. The two 10m transects perpendicular on the North- and South-transect, can either be placed North or South of the 10m transects. Insert for one of the 10m transects with the five locations where the height of the field layer was measured.



### Text A.3. Estimating effective detection distance

The effective detection distance (EDD) was estimated based on a model including all wildlife observations, as well as body mass estimates for each of the observed species, using a hazard rate detection function (Table A.1). Body mass estimates from the EltonTraits database (Wilman et al., 2014) were used.

Table A.1. Estimates of effective detection distance (EDD)

<i>Species</i>	<i>Body mass (kg)</i>	<i>EDD estimate</i>
Roe deer ( <i>Capreolus capreolus</i> )	22.5	7.64
Fallow deer ( <i>Dama dama</i> )	52.4	10.13
Wild boar ( <i>Sus scrofa</i> )	96.1	12.17
Red deer ( <i>Cervus elaphus</i> )	165.0	14.04
Moose ( <i>Alces alces</i> )	357.0	16.39

### Literature cited in Supplementary file S1

Spitzer, R., Churski, M., Felton, A., Heurich, M., Kuijper, D.P.J., Landman, M., Rodriguez, E., Singh, N.J., Taberlet, P., van Beeck Calkoen, S.T.S., Widemo, F., Cromsigt, J.P.G.M., 2019. Doubting dung: eDNA reveals high rates of misidentification in diverse European ungulate communities. *Eur. J. Wildl. Res.* 65, 28.

Stewart, K.E.J., Bourn, N.A.D., Thomas, J.A., 2001. An evaluation of three quick methods commonly used to assess sward height in ecology. *J. Appl. Ecol.* 38, 1148–1154.

Wilman, H., Belmaker, J., Simpson, J., de la Rosa, C., Rivadeneira, M.M., Jetz, W., 2014. EltonTraits 1.0: Species-level foraging attributes of the world's birds and mammals. *Ecology* 95, 2027.