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► To cite this version:

Mathieu Magnin, Jeanne-Marie Bonnet-Garin, Chiara Laurenza, Caroline Didier, Morgane Gavet, et al.. Evaluation of pimobendan effect on sublingual microcirculation in an experimental pharmacology induced hypotension porcine model. *Research in Veterinary Science*, 2022, 148, pp.7-14. 10.1016/j.rvsc.2022.03.021 . hal-04017288

HAL Id: hal-04017288

<https://vetagro-sup.hal.science/hal-04017288>

Submitted on 22 Jul 2024

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1 **Evaluation of pimobendan effect on sublingual microcirculation in an**
2 **experimental pharmacology induced hypotension porcine model**

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27 **Source of support:** This work was supported by VetAgro Sup - Veterinary Campus of Lyon, University
28 of Lyon, France. Pimobendane was donated by Boehringer Ingelheim.

29

30 **Abstract**

31 New therapeutic approaches are needed to simultaneously resuscitate macro- and
32 microcirculation during circulatory shock. The aims of this study were to explore the
33 microcirculatory and macrocirculatory effects of pimobendan, an inodilator with dual
34 phosphodiesterase 3 inhibitor and calcium-sensitizing effects, in an experimental porcine
35 model of pharmacologically induced hypotension associating vasoplegia and decreased
36 cardiac output. Eight piglets were anesthetized and monitored for their hemodynamic
37 parameters. Hypotension was induced by sevoflurane overdose until a mean arterial
38 pressure between 40 and 45 mmHg was reached. A bolus of pimobendan (0.25 mg/kg) was
39 administered intravenously thereafter. Sublingual microcirculation was evaluated using a
40 Sidestream Dark Field imaging device. Hemodynamic and microcirculatory parameters were
41 recorded at the baseline period (A), immediately before pimobendan administration (B) and
42 after pimobendan administration (C). Induction of hypotension was associated with a
43 decreased cardiac index and microcirculation alterations. Pimobendan administration was
44 associated with a significant increase in heart rate, cardiac index and decrease in systemic
45 vascular resistance index. A significant increase in proportion of perfused vessels for all
46 vessels (+8%, [2; 14], P = 0.01) and small vessels (+8% [1; 14], P = 0.03), in microvascular flow
47 index (+0.31 AU, [0.04; 0,58], P = 0.03) were noticed, as well as a decrease in heterogeneity
48 index (-0.34 [-0.66; -0.03], P = 0.04) and De Backer score for all vessels (-1.04, [-1.82; -0.25],
49 P = 0.02). In conclusion, in a simple model of pharmacologically induced hypotension,
50 pimobendan was associated with an improvement in several microcirculatory parameters.

51 Keywords:

- 52 - Microcirculation
- 53 - Phosphodiesterase type 3 inhibitor
- 54 - Calcium sensitizer
- 55 - Inodilators
- 56 - Pig

57

58 TEXT**59 1. Introduction**

60 In patients with circulatory failure, organ perfusion is often altered as a result of a low
61 cardiac output and / or arterial pressure, which has led to consider systemic hemodynamic
62 parameters as targets for patient resuscitation (Cecconi et al., 2014). However, tissue
63 perfusion can remain altered despite the restoration of within-target cardiac output and
64 arterial blood pressure (Hernandez et al., 2012; Lima and Bakker, 2014). This paradox,
65 known as hemodynamic incoherence, is probably due to the occurrence of microcirculation
66 alterations in the time-course of circulatory shock (De Backer et al., 2002; Trzeciak et al.,
67 2008). Hence, impairment in microvascular perfusion has been reported in several critical
68 conditions (De Backer et al., 2014). Based on this fact, it has been proposed to guide
69 resuscitation by targeting microcirculatory parameters in addition to the traditional systemic
70 hemodynamic ones (Ince, 2015; Kiyatkin and Bakker, 2017). If fluid therapy and
71 norepinephrine administration remain the first line treatment to restore perfusion pressure,
72 the occurrence of myocardial dysfunction in certain conditions, such as sepsis, requires some
73 inotropic support in addition. Sepsis-induced cardiomyopathy is a reversible myocardial

74 dysfunction associated with poor outcome and increased mortality (Antonucci et al., 2014;
75 Kakihana et al., 2016). Many questions persist regarding the appropriate treatment. Indeed,
76 the use of dobutamine allows the correction of heart dysfunction but does not reduce
77 mortality (Sato and Nasu, 2015). Levosimendan possessed interesting pharmacological
78 properties but was ultimately found to be disappointing in septic shock (Gordon et al.,
79 2016). Research for an effective treatment is therefore still relevant.

80 Pimobendan is a drug licensed for veterinary use indicated for the treatment of heart
81 failure in dogs. It is also used in Japan in human patients (Kawano et al., 2014; Yoshikawa et
82 al., 2000). It is a selective inhibitor of phosphodiesterase type 3 with calcium-sensitizing
83 effect, which induces both positive inotropism and vasodilatory action (Boyle and Leech,
84 2012a). These different properties make pimobendan an interesting option for inotropic
85 support and microcirculation resuscitation during shock and particularly septic shock.

86 To our knowledge, the microcirculatory effects of pimobendan have not been studied.
87 Consequently, the objectives of this study were to explore the microcirculatory and
88 macrocirculatory effects of pimobendan in a simple experimental porcine model associating
89 vasoplegia and decreased inotropism induced pharmacologically. Our hypothesis is that this
90 drug could allow, thanks to its inodilator properties, an improvement of the microcirculation
91 while preserving the macrocirculation.

92

93 **2. Materials and methods**

94 • *Ethical statement*

95 This study was conducted in accordance with the Guide for the Care and Use of
96 Laboratory Animals and approved by the Ethics Committee of our institution - Ethical
97 agreement: authorization number 1819.

98

99 • *Animals*

100 For the purpose of the study, 8 female piglets (30 – 60 kg) were anesthetized and
101 equipped for hemodynamic monitoring. Before the experiment, they were housed for a 7
102 day-acclimatization period in an individual box. They were fed with standard food, with *ad*
103 *libitum* access to water. Eight hours before the beginning of the experimental phase, solid
104 food was withdrawn while free access to water was allowed.

105

106 • *Anesthesia and equipment*

107 The anesthetic protocol consisted in a premedication with 3.0 mg/kg intramuscularly
108 (IM) of a 1:1 mixture of tiletamine-zolazepam (Zoletil100, 100 mg/mL, Virbac, Carros,
109 France), associated with 0.2 mg/kg IM morphine (Morphine aguetant, 10 mg/mL,
110 Laboratoire Aguetant, Lyon, France), followed by an induction with 4.0 mg/kg intravenous
111 (IV) propofol (Propovet 10 mg/mL, Zoetis, Malakoff, France), and a maintenance with
112 sevoflurane (Sevoflo, Zoetis, Malakoff, France) in 30% oxygen. Once anesthetized, the
113 external right jugular vein was dissected for placement of a central venous catheter
114 (Multicath 3 - 7.5Fr, Vygon, Ecoen, France). A pulmonary arterial catheter (Edwards Life
115 science catheter, Irvine, USA) was inserted in the right jugular vein for cardiac output (CO)
116 measurements. The suitable location of the catheter tip was determined by the
117 characteristic of the pressure curve.

118

119 • *Study design*

120 After instrumentation and stabilization of the animal, an initial fluid bolus (Ringer
121 Lactate, 10 mL.kg⁻¹ over 20 minutes) was administered to warrant the absence of preload
122 dependence. Then, a systemic hypotension was induced by sevoflurane overdose, with a
123 targeted mean arterial pressure (MAP) between 40 and 45 mmHg. Hypotension was
124 maintained over 45 minutes. Following the hypotension period, pimobendan (Vetmedin
125 Injectable, 0.75 mg/mL, Boehringer Ingelheim, Lyon, France) was administered at a dose of
126 0.25 mg/kg IV (0.33 mL/kg) over 10 minutes. Hemodynamic and microcirculatory parameters
127 were recorded at the baseline period (A, after the initial fluid bolus), immediately before
128 pimobendan administration (B, at the end of the hypotension period) and after pimobendan
129 administration (C). All parameters were recorded simultaneously when the animal appeared
130 hemodynamically stable over a period of 5 minutes.

131

132 • *Hemodynamics monitoring*

133 The following cardiovascular parameters were registered before and after
134 administration of pimobendan: heart rate (HR), mean arterial pressure (MAP) and CO
135 (L/min) by thermodilution. Cardiac index (CI, L/min/m²), stroke volume index (SVI, mL/m²)
136 and systemic vascular resistance index (SVRI, HRU/m² (hybrid resistance unit)) were
137 calculated based on conventional equations (CI = CO/BSA (body surface area, BSA (m²) =
138 0.0734 x body weight (kg)), SVI = CI/HR, SVRI = MAP/CI).

139

140 • *Microcirculation imaging*

141 Sublingual microcirculation was evaluated using a Sidestream Dark Field imaging
142 device (Microscan, Microvision medical, Amsterdam, The Netherlands). Five videos with a

143 standardized duration of 120 frames were recorded for each time period, as recommended
144 in the second consensus concerning the analysis of sublingual microcirculation (Ince et al.,
145 2018). The videomicroscope was connected to a computer using AVA software (Microscan,
146 Microvision medical, Amsterdam, The Netherlands, Version 4.3C).

147 Every video clip was recorded by the same trained investigator. All the videos were
148 recorded at the sublingual area. Briefly, before the introduction of the probe in the oral
149 cavity, the tongue was slightly raised, allowing the visualization of the lingual frenulum; the
150 probe was then placed gently in contact with the mucosa of the floor of the mouth and close
151 to the lingual frenulum, at an angle of about 60°. The recording procedures followed
152 Trzeciak and colleagues recommendations (Trzeciak et al., 2007).

153

154 • *Video clip analysis*

155 As recommended, only good quality videos were analyzed, based on a
156 microcirculation image quality score < 10 (Massey et al., 2013)). The microcirculation
157 parameters studied were as follows:

158

159 ■ Proportion of Perfused Vessels (PPV, %) and the De Backer score (DBs, n/mm)
160 were computed by the software. These parameters were calculated for all
161 vessels (all) and for small vessels (small), characterized by a diameter below
162 20 µm.

163 ■ The Microcirculatory Flow Index (MFI) was determined by a blinded evaluator
164 using quadrant-based MFI calculations. For this purpose, each video was
165 divided in four quadrants. A score was assigned by the evaluator for each
166 quadrant according to the predominant type of flow. The number assigned

167 was a subjective semi-quantitative flow value: 0 for no flow, 1 for intermittent
168 flow, 2 for sluggish flow, and 3 for continuous flow. The MFI score was the
169 average of these four values.

- 170 ■ Heterogeneity index (HI) was obtained by calculating the difference between
171 the highest quadrant MFI and the lowest quadrant MFI, divided by the MFI.
- 172 ■ The number of vessels crossing the 3x3 De Backer grid (NC) were also noticed
173 for small vessels (NC small, diameter < 20 μm) and for large vessels (NC large,
174 diameter > 20 μm). The AVA software calculates the number of crossings for
175 the vessels whose diameter is less than 20 μm and the number of crossings
176 for all the vessels observed. The number of crossings for large vessels (with a
177 diameter > 20 μm) was calculated by subtracting the number of crossings of
178 small vessels from that of all vessels.

179

180 PPV and MFI are determinant of red blood cell velocity (flow or no flow for PPV and
181 semi quantitative velocity for MFI) and are considered as parameters evaluating convective
182 capacity of the microcirculation. DBs is determinant of the number of vessels and is
183 considered as a parameter evaluating diffusive capacity of the microcirculation.

184

185 • *Statistical analysis*

186 All the variables were presented in median (25% quartile – 75% quartile). In order to test
187 the evolution of microcirculatory parameters, linear mixed models were computed for PPV,
188 MFI, HI, DBs and NC. They included the time period (A or B, B or C) as fixed effect and
189 individuals as random effects. For each model, the regression coefficient (estimate), the 95%
190 confidence interval (CI95) of the estimate and the value of P were presented. The

191 comparisons of the macrocirculatory variables (HR, MAP, CI, SVRI, SVI) were performed
192 using Wilcoxon signed-rank test. To test the influence of hemodynamic parameters on the
193 microcirculation evolution, a multivariate linear mixed model was built for all
194 microcirculatory variables. The complete model included CI, MAP and SVRI as fixed effects
195 and individuals as random effect. With the dredge function of the “MuMin” package, the
196 corrected Akaike criterion (AICc) was calculated for all possible sub-models. For each of the
197 microcirculatory variables studied, the model selected that better explain microcirculation
198 evolution corresponded to the most parsimonious model among those presenting the
199 lowest AICc ($\Delta AICc = 2$) (Mazerolle, 2006).

200 Statistical analysis was performed using R 3.5.2 software (R Foundation for Statistical
201 Computing, Vienna, Austria). The packages “ggplot2”(Wickham, 2009), “Lme4”(Bates et al.,
202 2015), Lmertest”(Kuznetsova et al., 2017), “MuMIn”(Burnham and Anderson, 2002),
203 “dplyr”(Wickham et al., 2020) and “gridExtra”(Auguie and Antonov, 2017) were used. A P-
204 value of 0.05 was considered as level of significance.

205

206 **3. Results**

207 Examples of microcirculatory videos are presented in the supplementary data file.

208

209 *a. Macro and microcirculatory alterations associated with hypotension* 210 *induction*

211 Hypotension was associated with a significantly decreased CI (A: 1.2 L/min/m² (1.1 -
212 1.5), B: 0.8 L/min/m² (0.8 – 0.9), P = 0.04). The variations of HR (A: 107 bpm (97 – 118), B: 85
213 bpm (82 – 97)) , SVI (A: 9.7 mL (9.2 – 13.9), B: 8.9 (7.9 – 11.3)) and SVRI (A: 56.7 HRU/m²

214 (39.2 – 69.2), B: 49.9 HRU/m² (48.8 – 63.5) , SVI (A: 9.7 mL (9.2 – 13.9), B: 8.9 mL (7.9 –
 215 11.3)) were non-significant (respectively P = 0.21, P = 0.31, P = 0.74) (figure 1).

216 Hypotension was associated with a significant decrease in PPV (estimate = -12%, CI95
 217 = [-18; -6], P = 0.0003), PPV small (estimate = -11%, CI95 = [-19; -4], P = 0.003), MFI (estimate
 218 = -0.57 AU, CI95 = [-0.85; -0.29], P = 0.0002), DBs (estimate = -1.30 n/mm, CI95 = [-2.01; -
 219 0.55], P = 0.0001) and an significant increase in HI (estimate = +0.45 AU, CI95 = [0.12; 0.78],
 220 P = 0.009). The reduction of DBs small was not significant (estimate = -0.16 n/mm, CI95 = [-
 221 1.43; 1.08], P = 0.8). The evolutions of these parameters were presented in figure 2.

222

223 *b. Evolution of macrocirculatory parameters before and after*
 224 *administration of pimobendan*

225

226 Macrocirculatory parameters values are presented in table 1. All parameters were
 227 recorded between 14 and 30 minutes after pimobendan administration (median time was 20
 228 minutes). Following pimobendan, HR (B: 85 bpm (82 – 97), C: 99 bpm (85 – 113)), CI (B: 0.8
 229 L/min/m² (0.8 – 0.9), C: 1.3 L/min/m² (1.1 -1.3)) and SVI (B: 8.9 mL/m² (7.9 – 11.3), C: 12.6
 230 mL/m² (11.4 – 13.1)) increased significantly (P = 0.03, P = 0.02 and P = 0.02 respectively). A
 231 significant decrease in SVRI (B: 49.9 HRU/m² (48.8 – 63.5), C: 40.1 HRU/m² (32.3 – 44.6), P =
 232 0.01) was also observed. Graphically, MAP appeared stable. The comparison showed no
 233 significant difference (B: 43 mmHg (40 - 45), C: 48 mmHg (42 – 53), P = 0.14).

234

235 *c. Evolution of microcirculatory parameters before and after*
 236 *administration of pimobendan*

237

238 Microcirculatory parameters values are presented in table 2 as median (25% quartile
239 – 75% quartile). Table 3 presents the results of the linear mixed models allowing to compare
240 the values of the microcirculation parameters before and after administration of
241 pimobendan. The results presented correspond to the mean change for each parameter
242 after administration of pimobendan (estimate), the 95% confidence interval of the estimate
243 and the value of P.

244 There was an overall increase in variables associated with convective blood flow after
245 administration of pimobendan (figure 2), in particular PPV all (estimate = +8%, CI95 = [2; 14],
246 P = 0.01, table 3), PPV small (estimate = +8%, CI95 = [1; 14], P = 0.03, table 3) and MFI (P =
247 0.03, table 3). Parallely, HI appeared to be significantly decreased (P = 0.04, table 3).

248 Regarding the MFI, it remained stable (at 3/3) for 3 pigs, it decreased in two pigs and
249 increased for 3 pigs (figure 2). According to the model, the mean increase after
250 administration of pimobendan was +0.31 AU (CI95 = [0.04; 0.58]). Likewise, the HI remained
251 stable (at 0) in 3 pigs, increased in two pigs, decreased in 3 pigs. According to the model, the
252 mean reduction in HI after administration of pimobendan was -0.85 AU (CI95 = [-1.52; -
253 0.12]).

254 The variables associated with diffusive capacity appeared decreased (figure 2): DBs
255 all was significantly reduced (estimate = -1.04 n/mm, CI95 = [-1.82; -0.25], P = 0.02, table 3)
256 while the decrease of DBs small was not significant (estimate = -1.16 n/mm, CI95 = [-2.43;
257 0.14], P = 0.08, table 3). The NC small showed a non-significant decrease (estimate = -5 n,
258 CI95 = [-11; 1], P = 0.08, table 3) after administration of pimobendan (figure 3).

259

260 *d. Association between microcirculation and macrocirculation during*
261 *pimobendan administration*

262

263 All the selected models for the microcirculatory variables studied only included CI as
264 fixed effect (table 4). CI was significantly associated with PPV all, PPV small, MFI, HI and DBs
265 all.

266

267 **4. Discussion**

268

269 This current study showed that pimobendan induces an increase of convective
270 microcirculatory variables (PPV all, PPV small, MFI), a decrease of heterogeneity variable (HI)
271 and a decrease in one diffusive variable (DBs). In addition, the administration of pimobendan
272 caused increased cardiac output and lower systemic vascular resistance. The improvement
273 of microcirculation following pimobendane administration was best explained by models
274 that included only the variation of cardiac output as explanatory variable.

275 The objective of this study was not to assess the clinical benefit of pimobendan in the
276 treatment of hypotension associated with anesthesia. Its main objective was to explore the
277 microcirculatory effects of pimobendan, never studied before. It was a first step before
278 evaluating the interest of this molecule in a septic shock model. We chose to use a simple
279 model associating vasoplegia and decreased inotropism, two major hemodynamic
280 alterations that can be observed during septic shock (Russell et al., 2018). For this purpose,
281 we used sevoflurane overdose to induce hypotension. The aim was to induce
282 microcirculatory disorders following hypotension. Indeed, even if the microcirculation is

283 relatively hemodynamically independent of the macrocirculation (de Backer, 2013), it seems
284 that marked hypotension can be at the origin of microcirculatory disorders. A study carried
285 out on pigs showed that below a certain pressure threshold (between 60 and 80 mmHg), the
286 sublingual microcirculation, evaluated by the MFI, decreased in a linear fashion (López et al.,
287 2015). Sevoflurane is known to cause hypotension when administered as an overdose by
288 peripheral vasodilation and decreased inotropy, as in the current study (De Hert and
289 Moerman, 2015; Otsuki et al., 2010). However, this simplistic model does not pretend to
290 imitate the pathophysiological complexity of septic shock, as other it is a complex
291 pathophysiological process with immune and inflammatory responses associated.

292 Thus, the use of pimobendan was expected, based on its inodilator properties, to
293 improve microcirculation and preserve macrocirculation. The dose of pimobendan
294 administered was chosen based on a previous study carried out on conscious pigs (Duncker
295 et al., 1987) and corresponds to the dose currently recommended in veterinary medicine in
296 dogs (Boyle and Leech, 2012a).

297 At baseline, we observed an important interindividual variability concerning cardiac
298 index. Particularly, two pigs exhibited low cardiac index, whereas a pig had high cardiac
299 index. There was no experimental condition known by the authors that could explain this
300 variability. This variability could be of physiological origin, be the consequence of a variability
301 in the response to anesthetics or even in the measurement method. To limit the variability
302 linked to the measurement, we performed three measurements of cardiac output at each
303 time of interest. Such interindividual variability was described in the literature (Bajorat et al.,
304 2006; Marx et al., 2000). This variability was markedly reduced after induction of
305 hypotension before administration of pimobendan.

306 On the macrocirculatory aspect, the administration of pimobendan was associated with
307 an increased cardiac output as expected, with an increase in both heart rate and stroke
308 volume. Systemic vascular resistances were decreased but the mean arterial pressure
309 remained within acceptable values, probably due to the compensatory action of cardiac
310 output. Worsening of hypotension was not observed in this study after administration of
311 pimobendan. Other studies on pigs have described similar effect (Duncker et al., 1987;
312 Stubenitsky and Duncker, n.d.) and this inotropic effect is well known in dogs (Hori et al.,
313 2019). These effects could be of interest in the treatment of sepsis-induced cardiomyopathy.
314 Indeed, it has the advantage of possessing positive inotropic effects which do not increase
315 the oxygen consumption by the myocardium, as is the case with dobutamine (Boyle and
316 Leech, 2012b). In addition, it has anti-inflammatory effects which could also turn out to be
317 interesting (Boyle and Leech, 2012b; Yang et al., 2019). Levosimendan, a molecule currently
318 used and having similar properties to pimobendan, has shown an interest in the
319 management of cardiac dysfunction during sepsis (Yang et al., 2019). However, one study
320 reports hypotension and hemodynamic instability associated with its use during septic shock
321 (Gordon et al., 2016). Hemodynamic instability was not observed with the use of
322 pimobendan in our study.

323 Parallely to the macrocirculatory effects, an increase in convective microcirculatory
324 variables was observed after the administration of pimobendan. These results are in
325 agreement with those reported with levosimendan, a molecule with similar
326 pharmacodynamic properties. In a study performed on human patients with septic shock
327 (Morelli et al., 2010), microcirculation was evaluated with an Sidestream Dark Field imaging
328 device. The administration of a 24h infusion of levosimendan at the dose of 0.2 $\mu\text{g}/\text{kg}/\text{min}$
329 was associated with an increase of convective microcirculatory variables (MFI and perfused

330 vessel density that reflect also diffusive capacity) and a decreased heterogeneity of the flow
331 (Morelli et al., 2010). Similarly, an improvement in the perfusion of the gastric mucosa was
332 observed experimentally in dogs after administration of levosimendan (Schwarte et al.,
333 2005). The increase in cardiac output is probably the predominant mechanism that improves
334 microcirculation in the current study, as it allows the maintenance of a perfusion pressure in
335 small vessels. The associated vasodilatory effect may have also contributed to the observed
336 improvement of the microcirculation by maintaining the driving pressure of blood flow at
337 the entrance of the microcirculation (Morelli et al., 2010). It seems to us that the volume of
338 the drug administered was too small (0.33 mL/kg) to have participated significantly in the
339 increase in microcirculatory parameters.

340 Concerning the MFI, the reading of the results reported in tables 2 and 3 could appear at
341 first sight contradictory. In table 2, the median MFI was reduced after administration of
342 pimobendan whereas in table 3 the average change in MFI between time B and C was a
343 statistically significant increase. Indeed, the two tables provide different information of a
344 different nature: table 2 displays central tendency indicators (median) with an indicator of
345 dispersion (interquartile range), whereas table 3 presents the results of the linear mixed
346 models performed to compare the pre and post pimobendane values. the moderate drop in
347 the median value was not a representative indicator of the overall trend, as it was partly
348 linked to the presence of numerous ex-æquo at time B (5 pigs have an MFI value equal to 3).
349 Furthermore, a reduction in the interquartile range was noticed at time C, indicating that
350 there were fewer low MFI values at this time-point, which was confirmed by the results of
351 the linear mixed models.

352 The decrease in diffusive parameters observed in our study was unexpected, based on
353 the vasodilatory effect of pimobendan. However, a reduction of total vessel density (a

354 similar parameter of the DBs) was described after an infusion of levosimendan in a patient
355 on whom a left ventricular assist device was implanted (Hessler et al., 2018). This reduction
356 was more pronounced in small vessels. The authors' hypothesis for this reduction consisted
357 in assuming that the small vessels were vasodilated consecutively to levosimendan and were
358 thus no longer counted as small vessels as vasodilatation increased their diameter. The
359 authors suggested that this observation could lead to a misinterpretation, as there was most
360 likely no microcirculatory dysfunction. Indeed, this patient did not present any sign of
361 alteration of the peripheral perfusion (absence of clinical signs of hypoperfusion and low
362 lactatemia). This assumption with levosimendan could be extrapolated to pimobendan and
363 explain the reduced DBs observed in our study since these two drugs share similar
364 vasodilating effects. In our study, we observed a decrease of systemic vascular resistance
365 (SVRI). Graphically, we observed a reduction in the number of small vessels, while the
366 number of larger diameter vessels seemed slightly increased but the differences were not
367 statistically significant. Considering these observations, it seems that the reduction in DBs all
368 and DBs small is probably consecutive to the reduction in the number of small vessels only.
369 Conversely, this reduction was not observed in a study performed in septic human patients,
370 where DBs was not different before and after the administration of levosimendan (Morelli et
371 al., 2010). Thus, further studies are needed to explore and confirm this hypothesis. If these
372 observations were confirmed on a larger scale, this would suggest that indicators such as
373 DBs or total vessel density are not reliable indicators for the evaluation of the
374 microcirculatory effects of inodilator molecules, such as levosimendan or pimobendan.

375 This study showed encouraging results for pimobendan as a supportive treatment of
376 systemic hemodynamic and microcirculation during circulatory failure. Nonetheless we must

377 remain cautious since contradictory results have been reported with levosimendan (Cholley
378 et al., 2019).

379 In addition, the present study has several limitations. The number of pigs limited the
380 statistical power of the current study. However, as it was an exploratory study, few
381 individuals were used for ethical reasons. In addition, in order to obtain a homogenous
382 population and reduce the number of animals, only females of similar age were included.
383 Therefore, the present results remain to be confirmed in a more heterogeneous population.

384 The measurements were performed on different sublingual areas for each period of the
385 study, whereas a continuous measurement would have allowed a better comparison over
386 time but was not possible with SDF technology. Indeed, the sublingual microcirculation
387 presents a certain heterogeneity. Nevertheless, we analyzed several videos at each time in
388 order to take into account this heterogeneity, as recommended in the consensus concerning
389 the analysis of sublingual microcirculation videos (Ince et al., 2018). We chose this method
390 for several reasons: firstly because it is this method that is used in clinical conditions,
391 secondly because continuous recording is not necessarily easy to achieve. Indeed, the quality
392 of the videos can vary over time, in particular because of the appearance of bubbles of saliva
393 or because of slight displacements of the tissues linked to the presence of saliva under the
394 microscope.

395 A continuous follow up of the hemodynamic parameters would have allowed to confirm
396 that the results obtained persisted over time. Unfortunately, this was not performed: the
397 hemodynamic measurements were carried out quickly after the administration of
398 pimobendan, when the parameters had reached a stable level, similarly to what has been
399 done in Duncker et al study (Duncker et al., 1987).

400 The most important limitation of this study concerns the simplicity of the experimental
401 model used. In this context we observe an improvement in microcirculation probably
402 associated with an increase in cardiac output. Such effects may not be found in a more
403 complex situation. In addition, other parameters aiming to quantify disorders of tissue
404 perfusion, such as lactates, diuresis or mixed venous oxygen saturation were not recorded
405 during the study.

406 This is an experimental study, which does not aim to give clinical recommendations. We
407 have chosen to use a simple model for exploratory purposes. Despite its limitations, this
408 model provided new information concerning a molecule which has not yet been studied
409 extensively. It allowed to observe that the administration of pimobendan was associated
410 with an increase of several microcirculatory parameters, with increased cardiac output and
411 was not associated with hypotension, as described after the administration of levosimendan
412 in septic shock in humans (Gordon et al., 2016). These encouraging results must therefore be
413 confirmed in a more complex model of septic shock. Another interesting observation is the
414 reduction in microcirculatory density, probably artefactual and secondary to vasodilation.
415 This phenomenon is not very well known and has already been observed in a patient with
416 levosimendan (Hessler et al., 2018).

417

418 In conclusion, in this study we described for the first-time the microcirculatory effects of
419 the administration of pimobendan, a phosphodiesterase type 3 inhibitor and calcium
420 sensitizer. In a simple model of pharmacology induced hypotension due to vasoplegia and
421 decreased cardiac output, pimobendan induced an increase of convective microcirculatory
422 variables and a decrease of a density variable, probably artefactual and secondary to
423 vasodilation. These results suggest a potential beneficial effect of pimobendan on sublingual

424 microcirculation. In this model, the microcirculatory improvement is probably consecutive to
425 an increase in cardiac output and to the vasodilatory property of pimobendan. Considering
426 the physiological abnormalities associated with septic shock (microcirculation and
427 myocardial dysfunction) pimobendan appears to be a potentially interesting treatment.
428 These encouraging results must be confirmed in a more complex model, such as a septic
429 shock model.

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434 5. References

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568 **6. Figures**

569 Figure 1. Evolution of macrocirculatory parameters

570 Legends: HR: heart rate, MAP: mean arterial pressure, CI: cardiac index, SVRI: systemic

571 vascular resistance index, SVI: stroke volume index. A: baseline, B: before pimobendan

572 administration, C: after pimobendan administration. The connected circles represent the

573 evolution of the parameters for each individual, * $P < 0.05$.

574

575 Figure 2. Evolution of microcirculatory parameters before and after administration of

576 pimobendan

577 Legends: The small dots represent each of the measurements. The connected circles

578 represent the medians of measurements by individuals. PPV: proportion of perfused vessels,

579 MFI: microvascular flow index, HI: heterogeneity index, AU: arbitrary unit, A: baseline, B:

580 before pimobendan administration, C: after pimobendan administration, * $P < 0.05$.

581

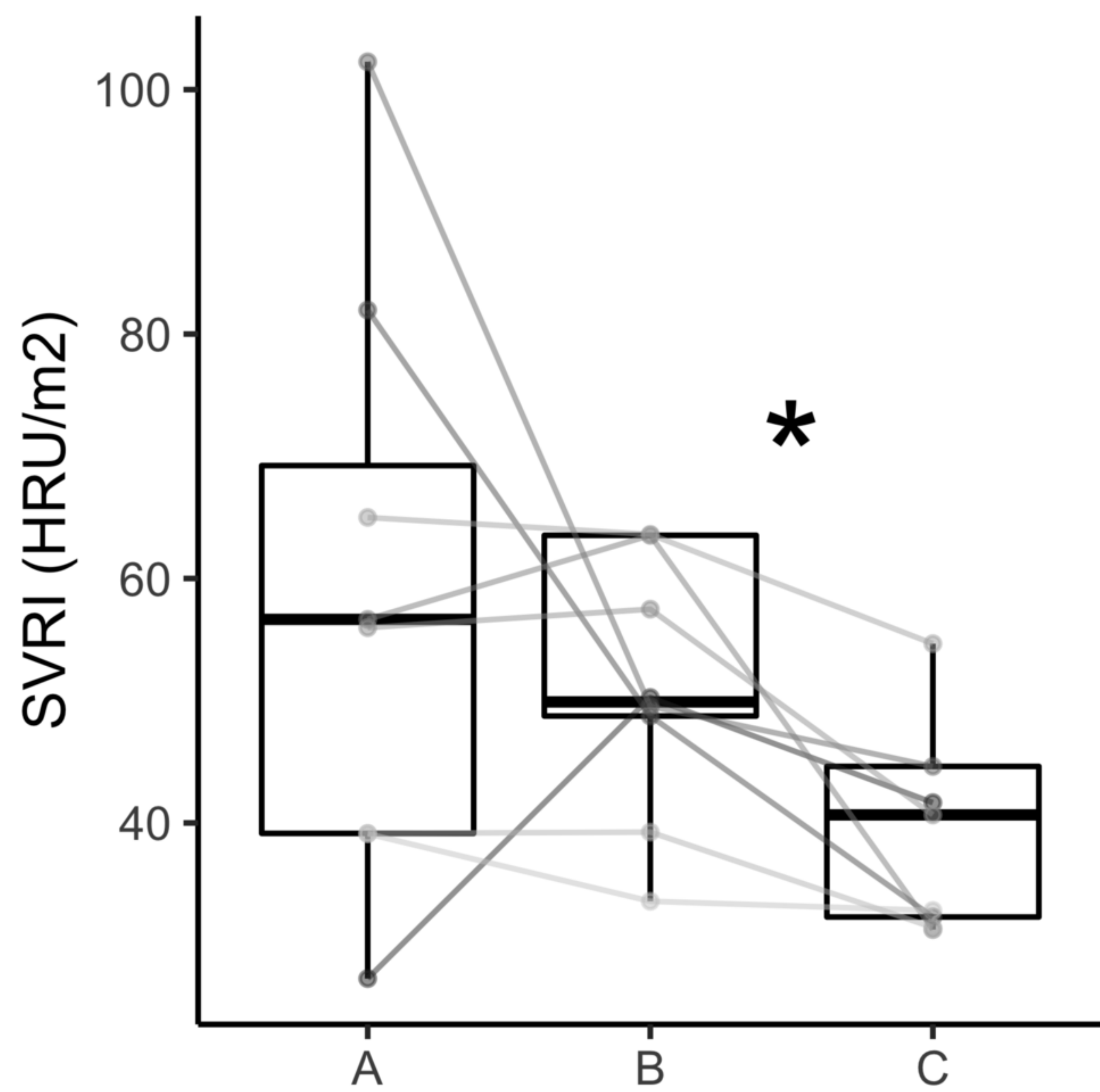
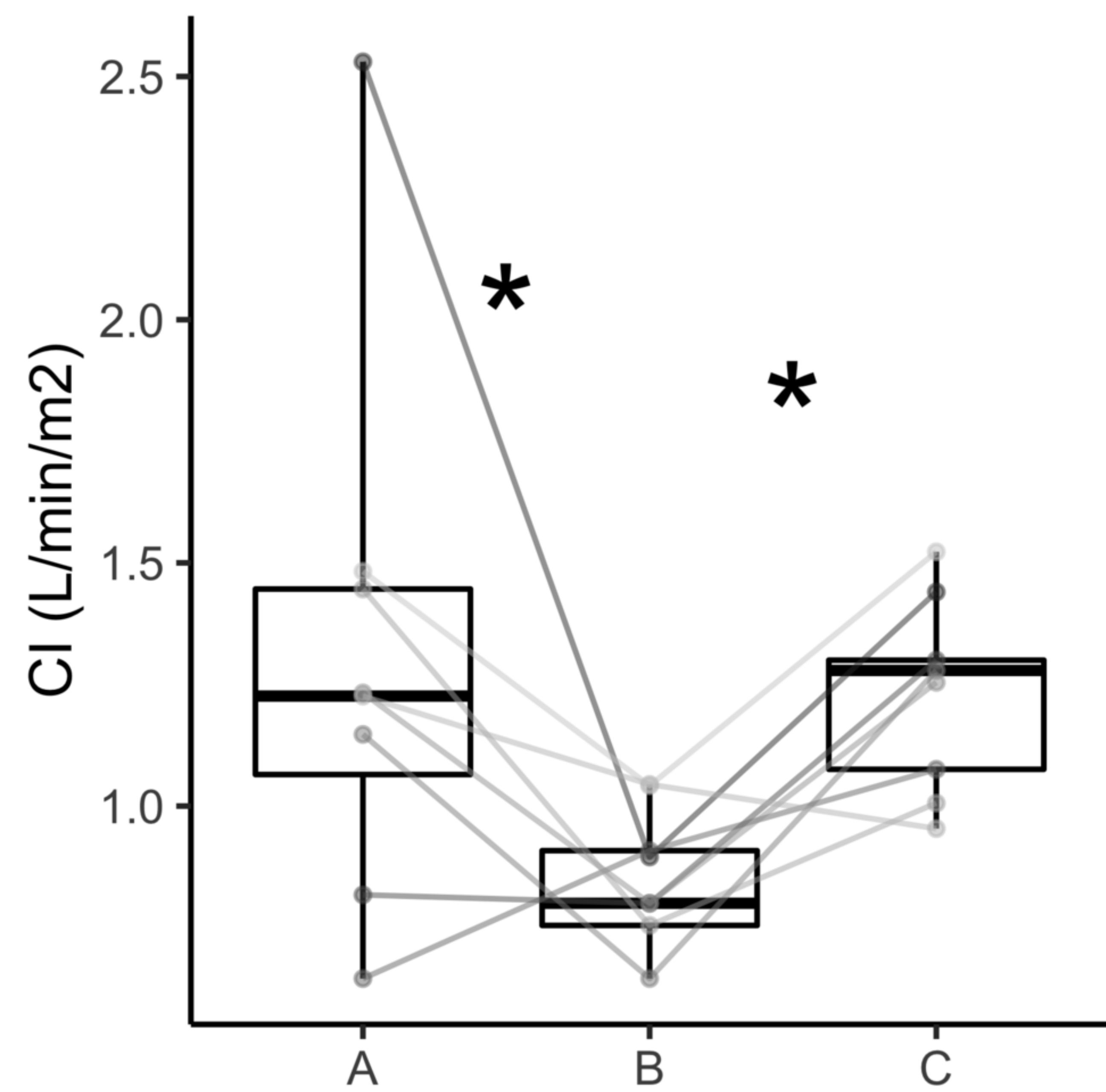
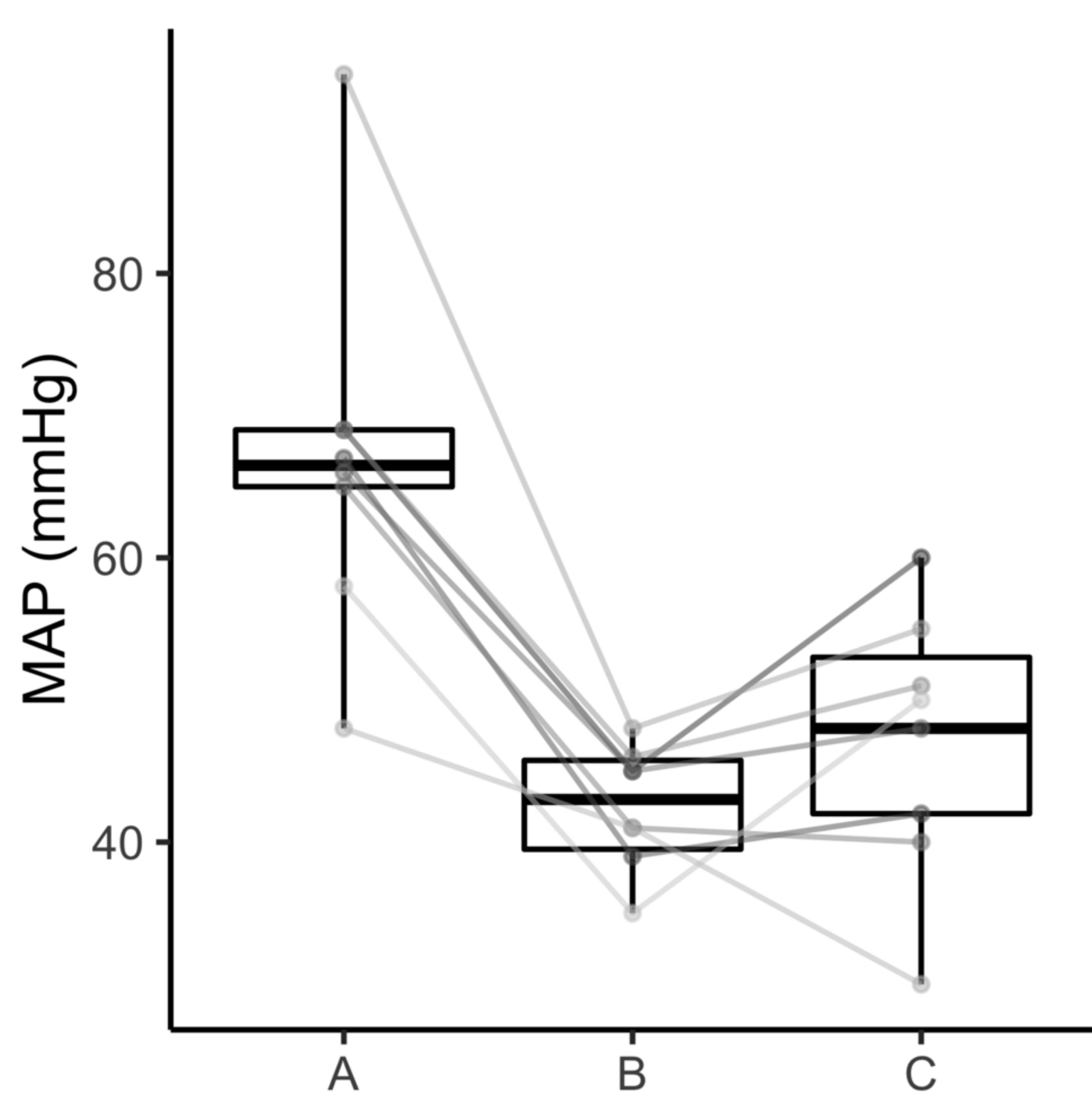
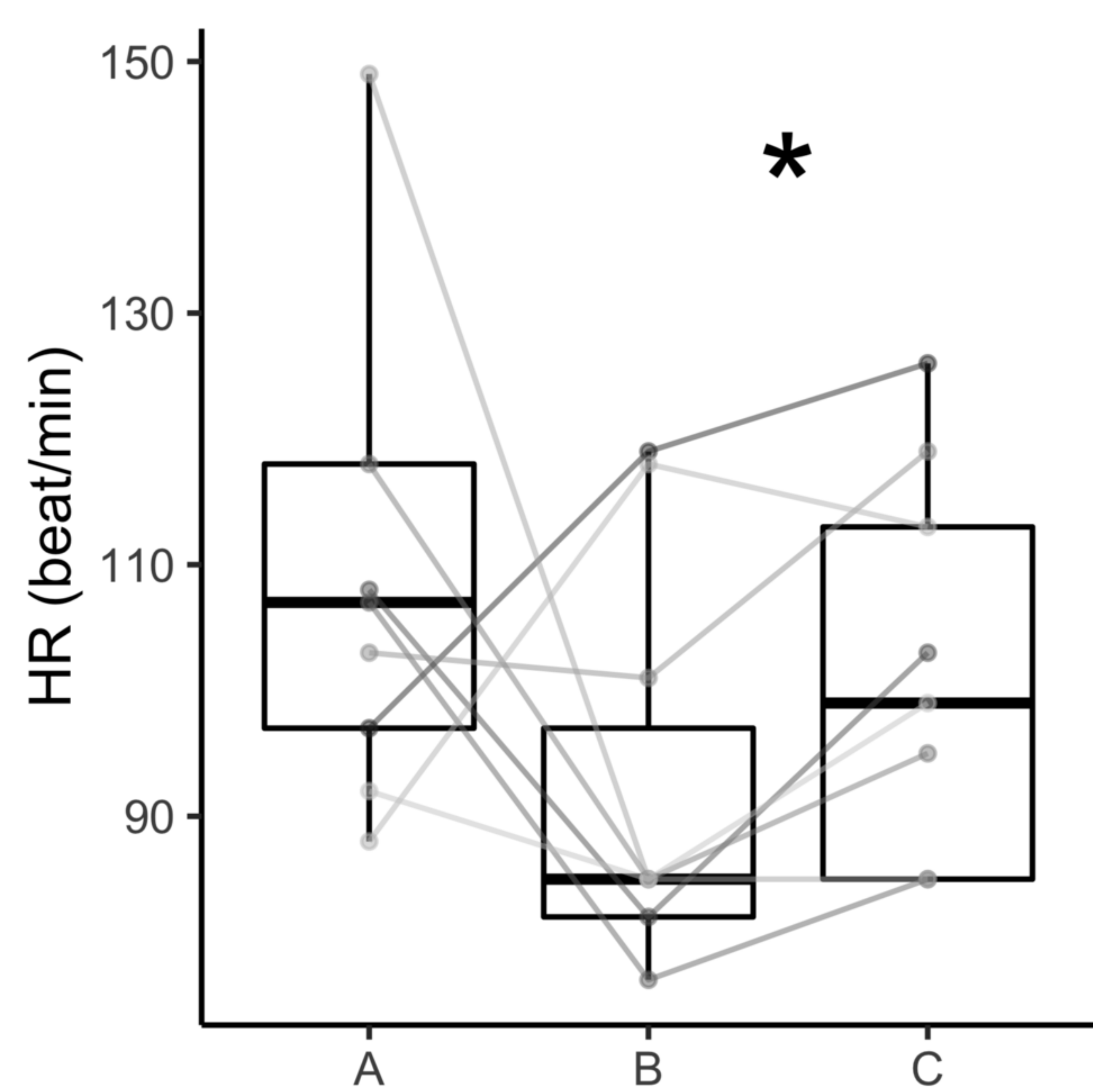
582 Figure 3. Evolution of the number of crossing vessels before and after administration of

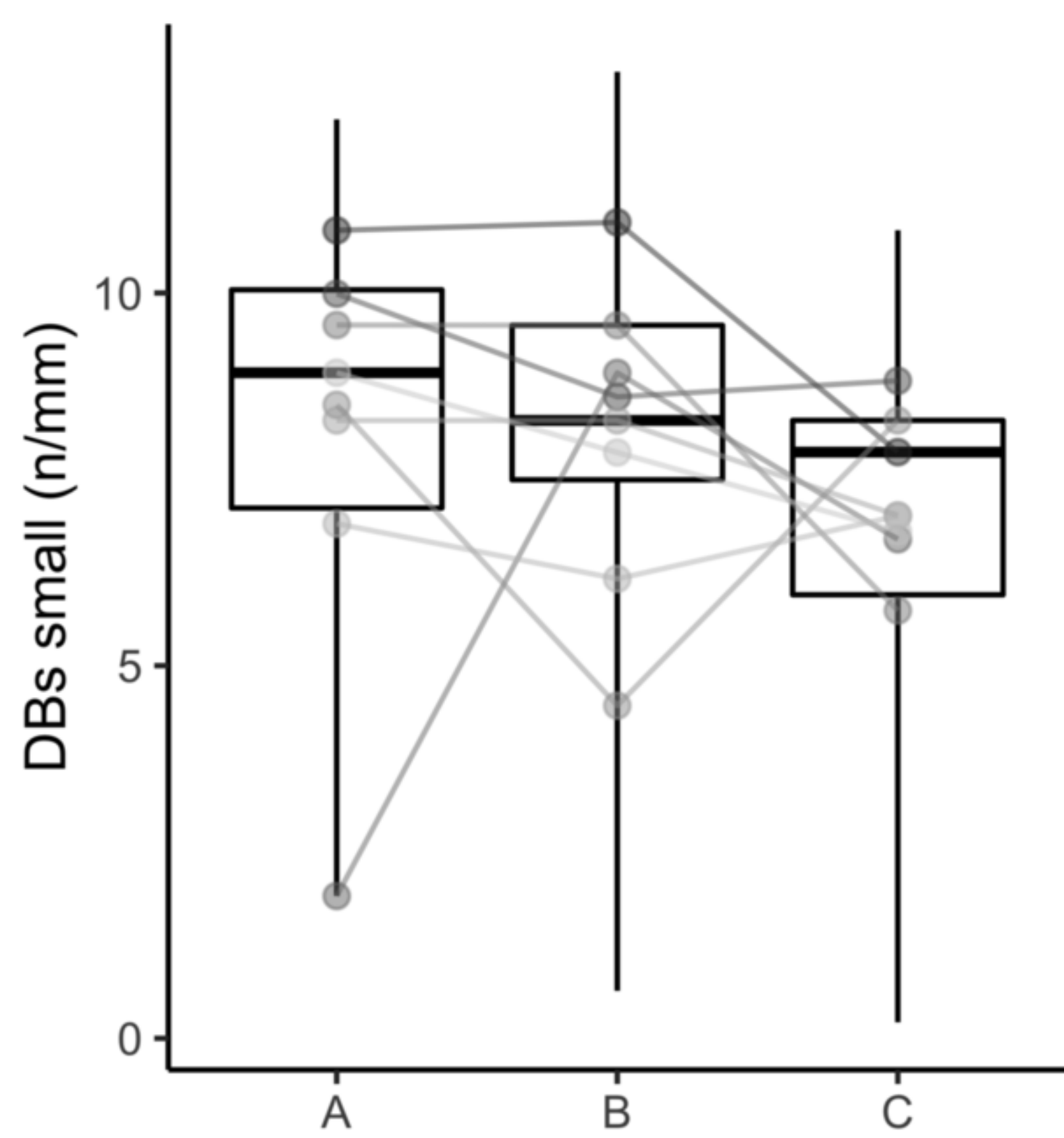
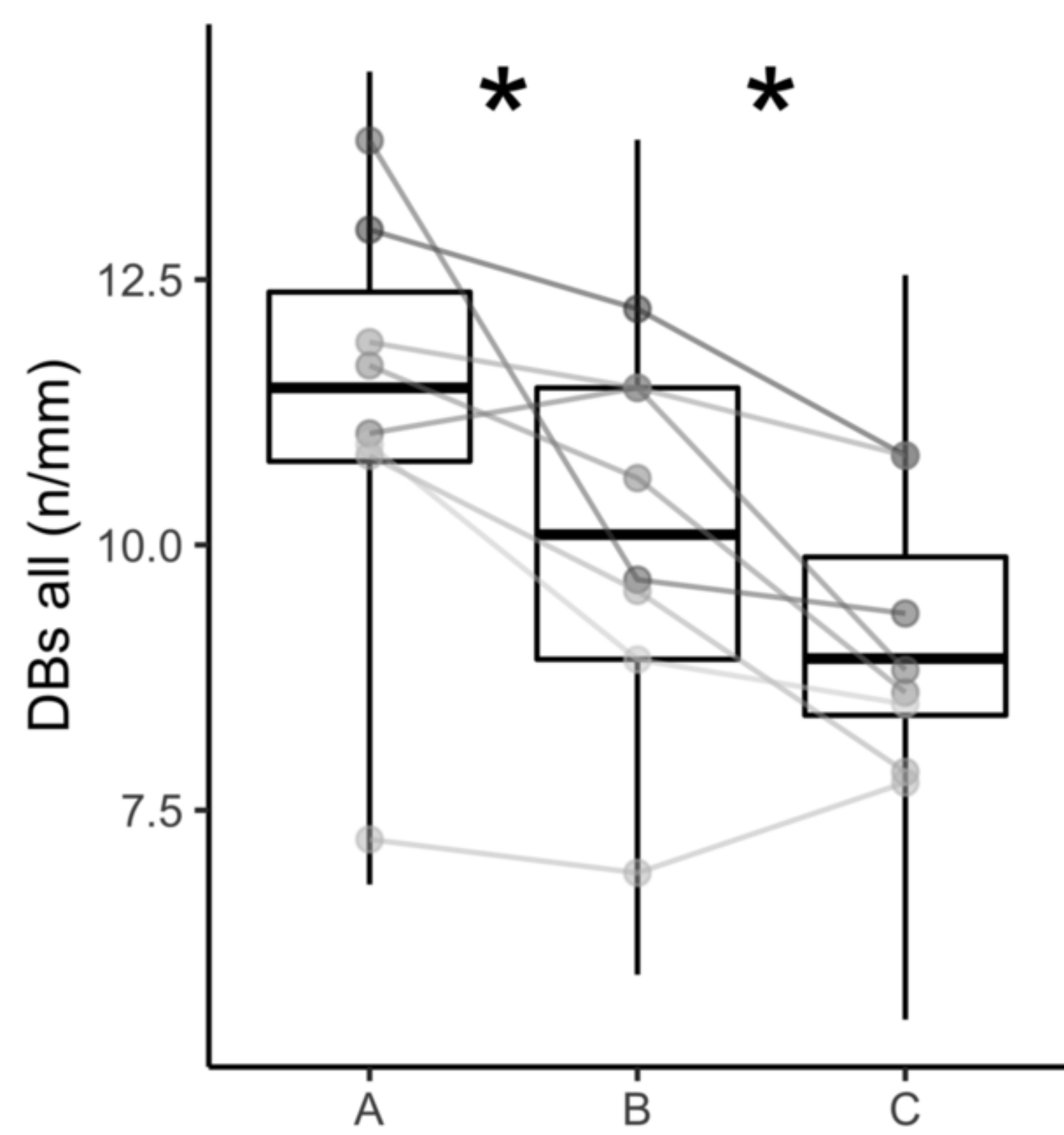
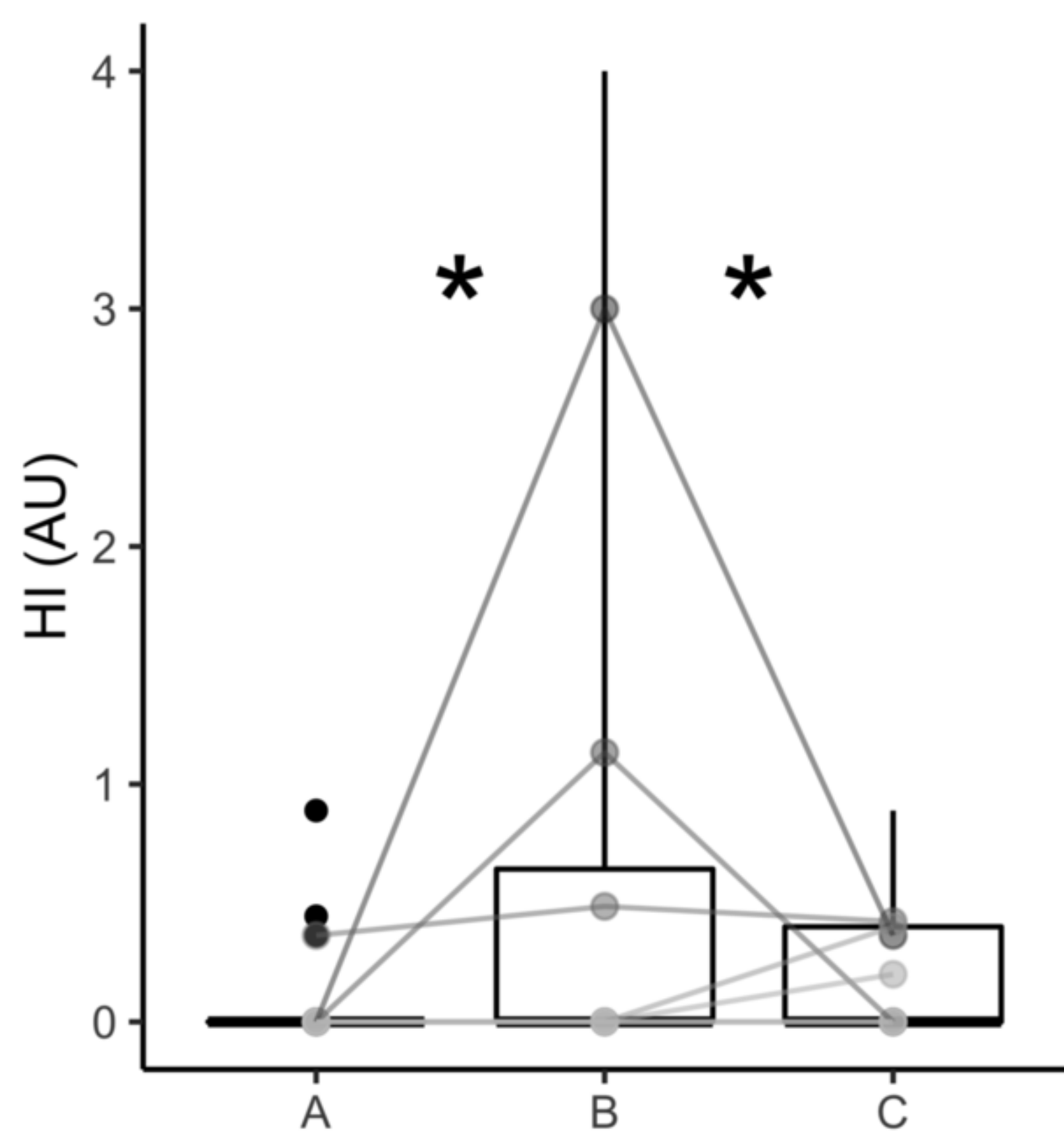
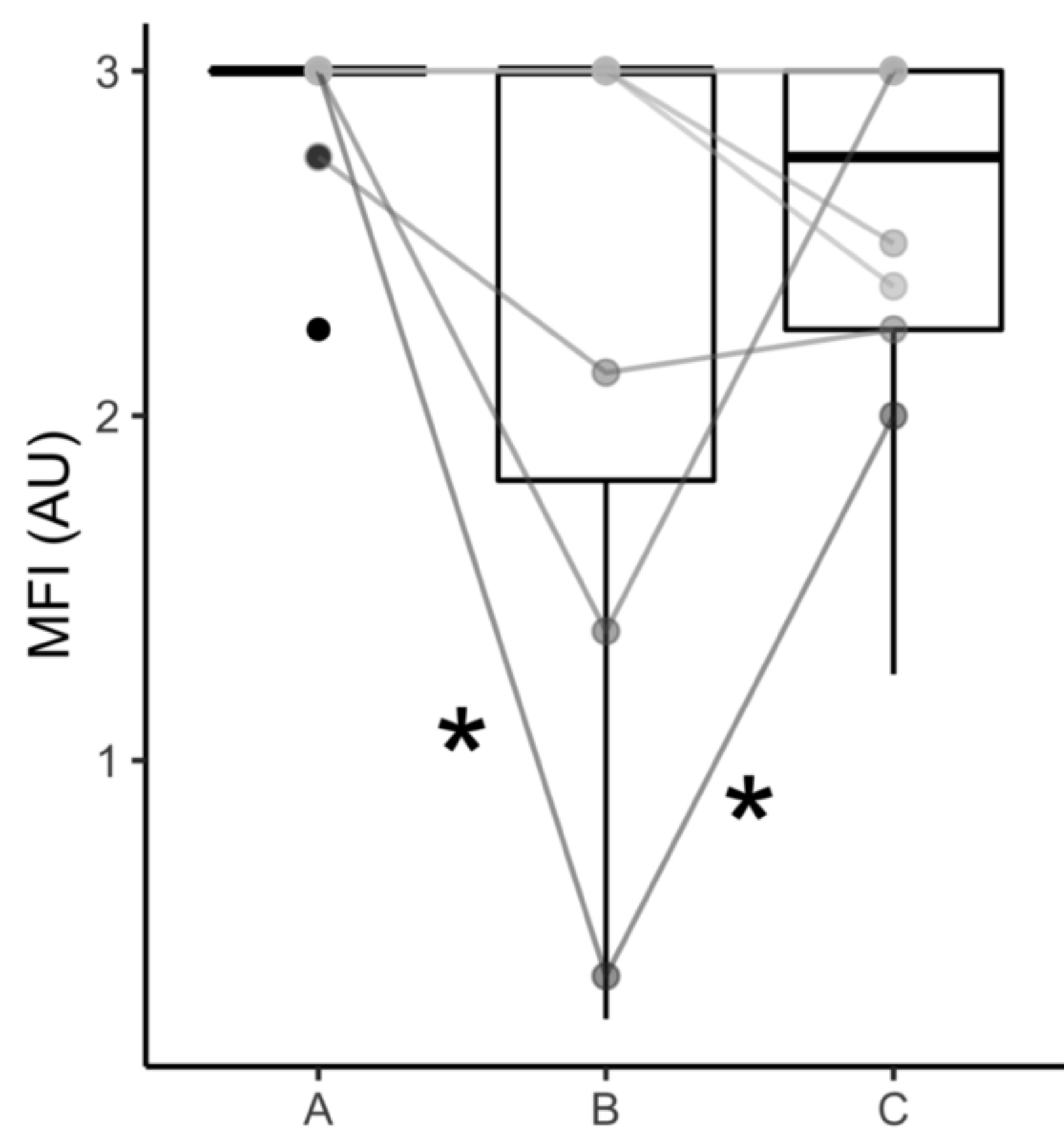
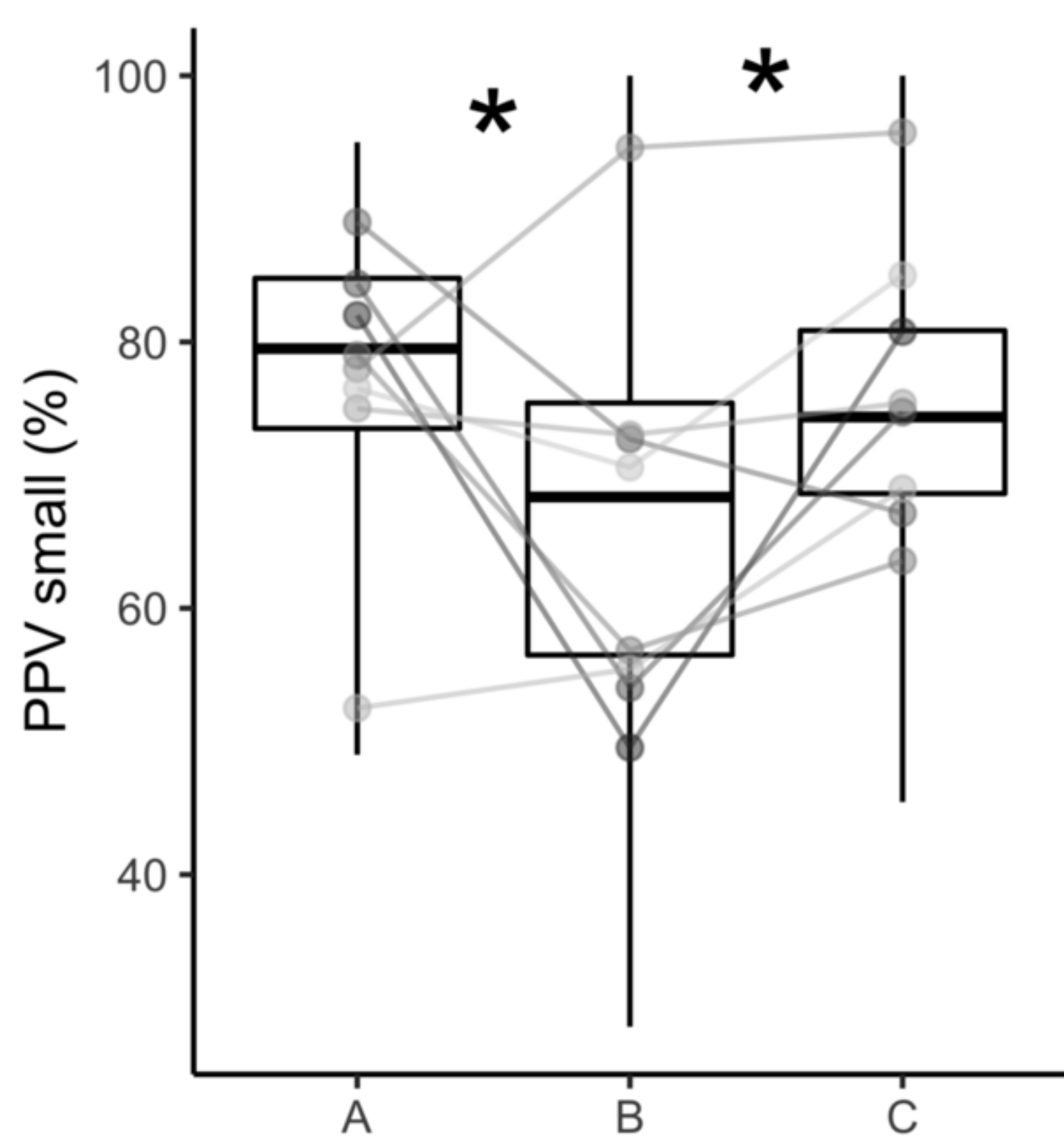
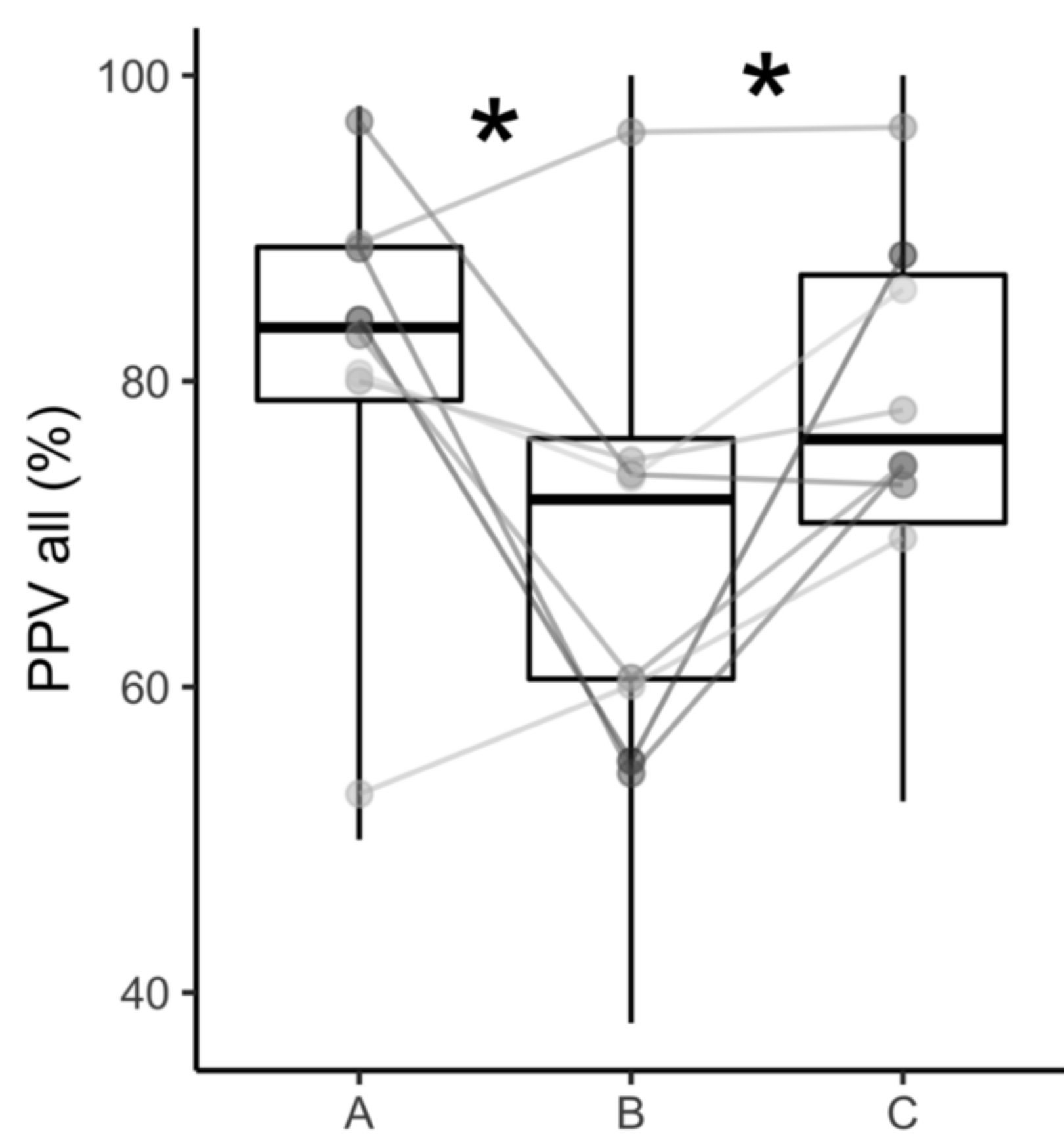
583 pimobendan

584 Legends: NC: number of crossings of large vessels (NC large) and small vessels (NC small), A:
585 before administration of pimobendan, B: after administration of pimobendan. The
586 connected circles represent the medians of measurements by individuals.

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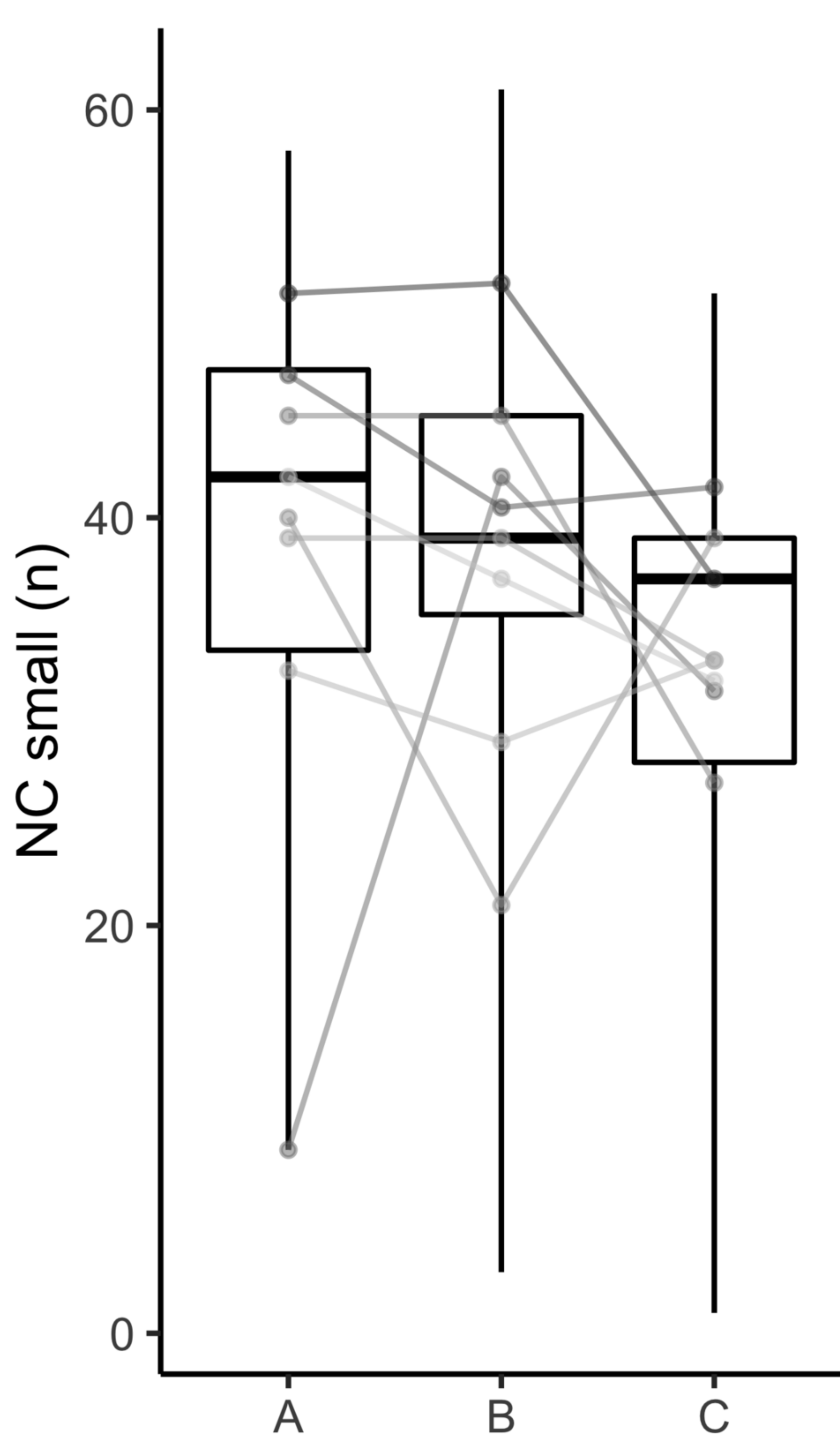
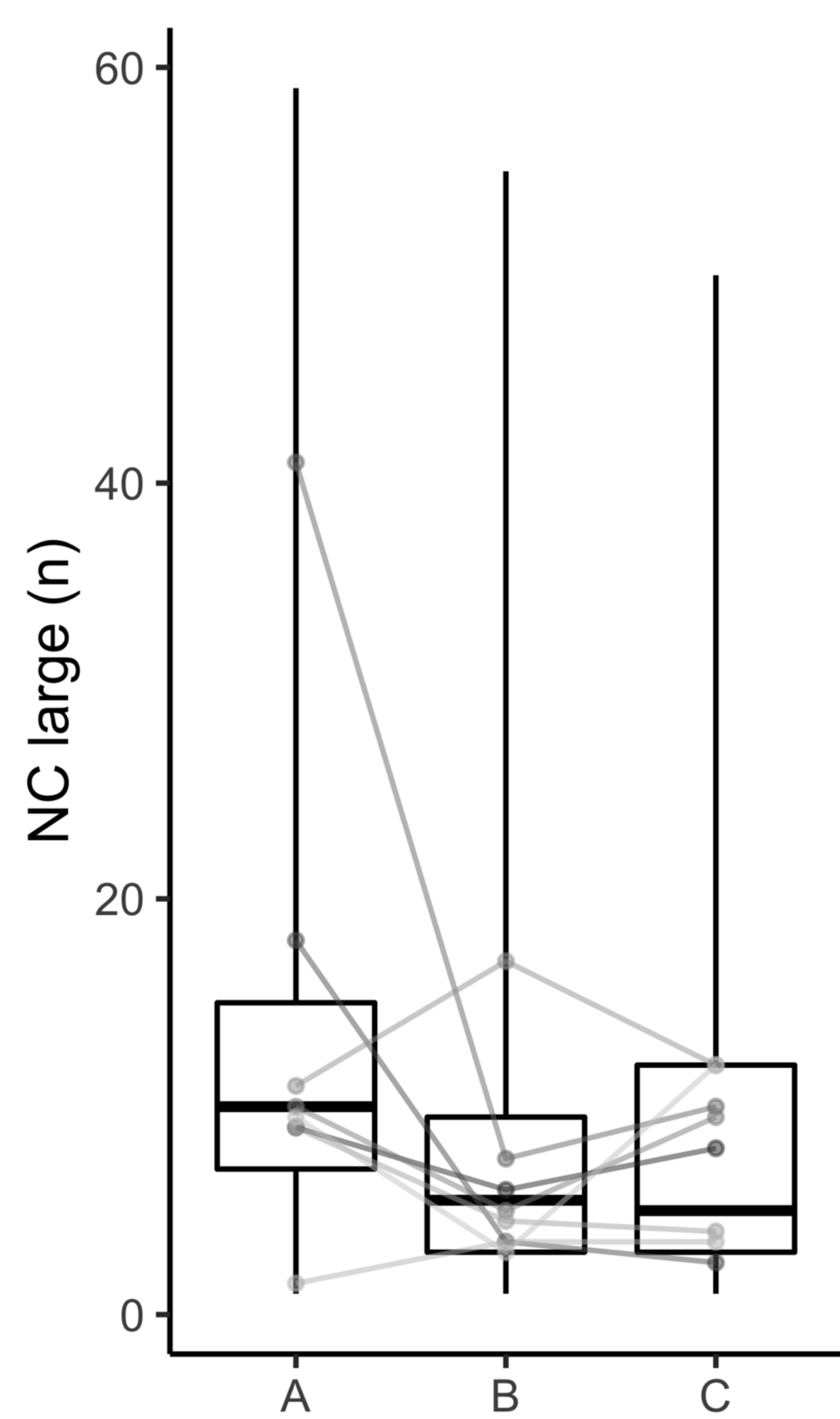


Table 1. Macrocirculatory parameters before and after administration of pimobendan

	Units	Before administration	After administration	P
HR	Beat/min	85 (82 – 97)	99 (85 – 113)	0.03
MAP	mmHg	43 (40 – 43)	48 (42 – 53)	0.14
CI	L/min/m ²	0.8 (0.8 – 0.9)	1.3 (1.1 – 1.3)	0.02
SVRI	HRU/m ²	50 (49 – 64)	41 (32 – 39)	0.01
SVI	mL/beat/m ²	9 (8 – 11)	13 (11 – 13)	0.02

HR: heart rate, MAP: mean arterial pressure, CI: cardiac index, SVRI: systemic vascular resistance index, SVI: stroke volume index.

Table 2. Microcirculatory parameters before and after administration of pimobendan

Microcirculatory variables	Units	Before administration	After administration
PPV all	%	72 (60 – 76)	76 (71 – 87)
PPV small	%	68 (56 – 75)	74 (69 – 81)
MFI	AU	3 (1.8 – 3.0)	2.8 (2.3 – 3.0)
HI	AU	0.0 (0.0 – 0.7)	0.0 (0.0 – 0.04)
DBs	n/mm	10.6 (9.4 – 11.5)	9.3 (8.5 – 10.2)
DBs small	n/mm	8.3 (7.5 – 9.6)	7.9 (6.0 – 8.3)
NC large	n	5.5 (3 – 9.5)	5 (3 – 12)
NC small	n	39 (35 – 45)	37 (28 – 39)

PPV: Proportion of Perfused Vessels, MFI: Microvascular Flow Index, HI: Heterogeneity

Index, DBs: De Backer score, NC large: number of crossings of large vessels, NC small:

number of crossings of small vessels, AU: arbitrary unit

Table 3. Impact of pimobendan administration on microcirculatory variables

Microcirculatory variables	Units	Estimate	95%CI	P
PPV all	%	+8	[2 ; 14]	0.01
PPV small	%	+ 8	[1 ; 14]	0.03
MFI	AU	+ 0.31	[0.04 ; 0.58]	0.03
HI	AU	-0.34	[-0.66 ; -0.03]	0.04
DBs	n/mm	-1.04	[-1.82 ; -0.25]	0.02
DBs small	n/mm	-1.16	[-2.43 ; 0.14]	0.08
NC large	n	+1	[-5 ; 6]	0.83
NC small	n	-5	[-11 ; 1]	0.08

PPV: Proportion of Perfused Vessels, MFI: Microvascular Flow Index, HI: Heterogeneity

Index, DBs: De Backer score, NC large: number of crossings of large vessels, NC small:

number of crossings of small vessels, AU: arbitrary unit, 95%CI: 95% confidence interval

Table 4. Association between microcirculation and macrocirculation

Microcirculatory variable	Macrocirculatory variable selected as fixed effect	Estimate	95%CI	P
PPV all	CI	19.9	[6.6 ; 32.8]	0.004
PPV small	CI	20.5	[4.9 ; 35.6]	0.01
MFI	CI	0.87	[0.26 ; 1.45]	0.005
HI	CI	-0.85	[-1.52 ; -0.12]	0.02
DBs all	CI	-2.1	[-3.8 ; -0.3]	0.02
DBs small	CI	-2.7	[-5.4 ; 0.0]	0.06

CI: cardiac index, PPV: Proportion of Perfused Vessels, MFI: Microvascular Flow Index, HI:

Heterogeneity Index, DBs: De Backer score, 95%CI: 95% confidence interval