

We thank the reviewers for the comments, and to editors for giving us a chance to address them and for their patience in waiting of our responses. We have left the reviewers comments in roman and added our responses in italics. Our modifications to the manuscript are shown as screenshots but we are also uploading a separate file where the modifications can be tracked directly. We hope that you will find the modifications adequate.

Kind regards,
Stanislav Jelavić

Review 1.

Comment:

The paper by Jelavic et al. focuses on the adsorption of DNA by two carbonaceous materials, namely soot and charcoal. The subject bears some environmental relevance and, in that regard, this could be an interesting study. Unfortunately, the manuscript in its present form is in my opinion scientifically rather poor and does not deserve publication.

General remarks.

The authors present a few adsorption isotherms of DNA on soot and charcoal with less than 10 data points per isotherm and then they compare these isotherms to various models, compute some kind of degree of merit and from the best fit, supposedly deduce adsorption mechanisms. Such an approach is extremely reductive and furthermore is scientifically unsound. The fact that isotherm data are described by a certain model does not provide any evidence that the assumptions underlying the model are true. There are hundreds of examples where, for instance, isotherms can be described by a Langmuir model whereas the basic assumptions of this model, i.e. monolayer adsorption, homogeneous adsorption energy and absence of lateral interactions are not fulfilled. Furthermore, the fact, which is the case here, that the Freundlich model provides a good fit does not provide any meaningful information about adsorption mechanisms. The fact that natural surfaces are energetically heterogeneous is a generality. It is truly a pity that the authors do not try to get more information from the data they gathered as I think that there are a few tendencies, notably concerning the role of the background electrolyte that could be useful.

Response:

To produce a reliable adsorption isotherm, the number of experimental points (initial DNA concentrations) ought to be higher than the number of parameters describing the data (2-4 times higher in our study), the distribution of experimental points ought to be large enough (maximum initial concentration is 80 times larger than minimum in our study) and each experimental point ought to be a replicate (triplicates in our case). Consequently, we maintain that our experimental design is sound, and do not

understand the the requirement for 10 “data points” per isotherm, as it appears somewhat arbitrary to us.

The reviewer is correct in pointing out that a successful fit of a sorption isotherm model to data does not infer anything about adsorption mechanisms. When we perform these fits, it is simply a way of quantifying differences between the processes at two solids, i.e. charcoal and soot, as well as between solution conditions such as pH. Simple comparison of isotherms “by eye” is imprecise in identification of various processes and “tendencies” from adsorption data. The reason we discuss the details of isotherm models with respect to adsorption data in our study is to emphasise the differences in adsorption of DNA at soot and charcoal as a function of solution composition and DNA length. We did not use modeling of adsorption isotherms to infer the adsorption mechanism of DNA at soot and charcoal. We used isotherm modeling to compare adsorption process of different lengths of DNA strands in presence of different cations, and their high and low concentrations. We then juxtaposed adsorption results to a) the composition of soot and charcoal determined by Raman and XPS, b) charging properties of soot and charcoal in electrolyte solutions determined by mass titration and zeta potential measurements and c) the hydrophobicity of soot and charcoal determined by water adsorption, to infer the material property controlling the adsorption and infer the likely adsorption mechanism. We have added a few sentences to clarify our approach:

87 function of pH, ionic strength, solution composition, time and DNA length. We used isotherm
88 modelling to quantify differences in isotherm shapes. By evaluating how the surface properties of soot
89 and charcoal influence the adsorption of DNA as a function of solution composition, we infer a likely
90 adsorption mechanism. We propose that, besides electrostatic forces, hydrophobic interactions play
91 an important role in adsorption of DNA to soot and charcoal. This information can be used for
92 improving protocols of eDNA extraction from environmental matrices where soot and charcoal are
93 abundant such as urban and wildfire aerosol, and topsoil. This is important because DNA adsorbed at
94 soot and charcoal could hold (paleo)biodiversity information that is not available through routine
95 eDNA extraction and analysis. Advancing our understanding of interactions between DNA and
96 environmental surfaces will provide an important contribution to understanding of eDNA reservoirs
97 in the environment.

200 **Modelling of equilibrium adsorption and kinetic data.** We fit the adsorption isotherms using
201 equations that model monolayer and multilayer adsorption (but acknowledge that such modelling
202 alone does not reveal how adsorption takes place in reality), and the kinetic data using equations that
203 model surface and diffusion -controlled processes (Table S1.). We applied nonlinear least squares
204 regression to fit data to models. We chose the **mathematically best fitting most-appropriate**-model by
205 comparing their reduced chi-squared parameter of fits, χ^2_v , i.e. the χ^2_v closest to 1 was considered the
206 best. If the best fit resulted in standard errors that were larger than the fitting parameters, the fit with
207 χ^2_v that was next in line but with standard errors smaller than the fitting parameters was considered
208 more appropriate, i.e., matching the form of curve better.

387 The fitting to isotherm models revealed very similar behaviour as for the short DNA: The adsorption
388 of long DNA in electrolytes is best ~~described~~^{explained} by a multilayer adsorption ~~model~~^{process} for
389 ~~an that happens at e~~energetically heterogeneous surface (quality of fit parameters in Table S6, model
390 fits in Figure 4A). A better fit of the isotherm for charcoal in pure water to ~~Temkin~~ rather than
391 ~~Freundlich~~ model suggest that there is either a uniform distribution of heterogeneous binding sites or
392 that there is interaction between ~~neighbouring~~ DNA molecules.⁵⁶ Long DNA adsorption at soot in pure
393 water is still best ~~explained~~^{described} by a monolayer adsorption ~~model~~^{but the adsorption sites are}
394 ~~energetically similar~~ (Langmuir model). This stands in contrast to ~~adsorption of short DNA that is best~~
395 ~~described by model for~~ monolayer adsorption ~~of short DNA~~ at heterogeneous surface (Sips model,
396 Table S3).

397 For long DNA, many of the tested models often fit the data well. Some fits had χ^2_v very close to 1 but
398 the value of standard deviation was larger than the fitted model parameters (red in Table S6). In these
399 cases, we considered as the best, the fit that had χ^2_v next in line but had standard deviation smaller
400 than the fitted model parameters. The fact that the fitting parameters do not give a conclusive picture
401 about the adsorption of long DNA suggests that the ~~mechanism~~^{adsorption process} is likely more
402 complicated than in the case of short DNA. However, we did observe that all models that closely fit
403 experimental data had similar assumptions and implications, *i.e.* adsorption of long DNA at soot in
404 pure water is similarly well fit with both Langmuir and ~~Toth~~ models (Table S6). Since the z parameter
405 of ~~Toth~~ model was ~ 1 , this suggests that the adsorption is ~~best described by a model for a~~ monolayer
406 process but ~~suggesting that the surface is heterogeneous. there might be more than one active site~~
407 ~~because of the good fit to the Langmuir model.~~

Detailed remarks.

Comment:

• Line 17. The statement that a comprehensive insight is lacking does not take into account the large body of literature on the subject including the various studies where nucleosides and nucleotides have been adsorbed on various environmental surfaces.

Response:

Correct, our statement does not consider those studies because adsorption of nucleotides and nucleosides at carbonaceous materials is only marginally relevant for the understanding of adsorption of DNA at soot and charcoal. In Lines 55-81, we do however give an overview of theoretical and experimental studies relevant for our system:

55 Knowledge of the binding between the DNA and CMs is important for understanding the adsorption
56 under various environmental conditions. Extracellular eDNA is principally double stranded DNA
57 (dsDNA) since this form is more resistant to degradation than single stranded DNA.^{18–21} Studies of the
58 interaction between dsDNA and materials compositionally and structurally similar to soot and
59 charcoal such as graphene, graphene oxide (GO) and reduced graphene oxide (rGO) have already
60 provided insight into the eDNA binding at CMs.^{22–24} Molecular dynamics simulation suggested that, at
61 oxygen-lacking CM's such as graphene, dsDNA binds to surface via the terminal base pairs through π –
62 π stacking.²⁵ dsDNA can bind either using only one termination, with the helix axis perpendicular to
63 the graphene surface (“standing up”), or with both terminations forming a horseshoe shape, with the
64 axis mostly parallel to the surface except close to terminations where base pairs are severely
65 deformed. From studies of oxygen-containing CM's such as GO and rGO, we know that dsDNA can
66 bind either electrostatically via the negatively phosphate backbone (helix axis parallel to adsorbent
67 surface - “lying down”) or by π – π interaction and hydrogen bonding via the base pairs at the end of
68 DNA,^{26–28} as with graphene. In the absence of electrolytes that reduce electrostatic repulsion between
69 negatively charged GO or rGO and negatively charged phosphate backbone, bulk adsorption studies
70 suggest that hydrophobic forces dominate the interaction with DNA.²⁹ However, in the presence of
71 electrolytes, electrostatic interaction becomes more important evidenced by increasing DNA
72 adsorption capacity as the ionic strength increases^{29,30} or as pH decreases.²⁹ The distribution of oxygen
73 functional groups in GO and rGO is highly heterogeneous,^{31,32} *i.e.*, they contain areas that are rich and
74 areas that are poor in functional groups. The interaction between these surfaces and the phosphate
75 backbone likely takes places at the areas rich in hydrophilic functional groups. In contrast, the π – π
76 stacking takes place at areas poor in oxygen functional groups (graphene-like). Combined, these
77 studies suggest that the ratio of hydrophilic and hydrophobic areas in carbonaceous materials
78 determines their overall interaction with dsDNA, with hydrophobic interactions becoming dominant
79 in materials rich in graphene-like surfaces. **However, graphene-like materials are rare in the
80 environment and it is unclear to which extent our current understanding of DNA interactions with
81 carbonaceous materials is applicable to environmentally common surfaces such as soot and charcoal.**

In addition, if we elaborate on nucleotide and nucleoside adsorption, it would be necessary to include the literature available on DNA-solids adsorption in general. We would like to keep the focus to DNA and carbonaceous materials.

Comment:

- Line 43. The term CM is not defined.

Response:

The term is defined two lines above, in Line 41.

- 41 mineral environmental surfaces such as carbonaceous materials (CM) to the environmental reservoir
42 of DNA is unclear.
- 43 CMs are produced anthropogenically and naturally by burning fossil fuels and vegetation. CMs are

Comment:

- Line 185 and after. See general remarks.

Response:

See our response above.

Comment:

• Line 194 and Figure 1A. Labelling the axis as 2θ without indicating the wavelength is somehow meaningless.

Response:

The wavelength is stated in the Materials and Methods section (Line 103). We do not think it is necessary to repeat the method details in figure captions.

100 We purchased carbon soot nanopowder (NANOSHEL, >98.9%, CAS: 7440-44-0), further called soot,
101 and activated charcoal (DARCO, Sigma Aldrich), further called charcoal. We used XRD to identify major
102 and minor contaminants. We collected diffractograms between 5-90 $^{\circ}2\theta$ using a Bruker D8
103 diffractometer equipped with Cu K_{α} radiation (40 kV, 40 mA; $\lambda \sim 1.543 \text{ \AA}$). We used step size of 0.04
104 $^{\circ}2\theta$, time per step of 6 s and spun the sample at 20 rpm with 0.3 $^{\circ}$ divergence and antiscatter slit and
105 2.3 $^{\circ}$ Soller slits on both incident and diffracted beams.

Comment:

• Line 202 and Figure 1B. The role of surface oxygen that could represent polar groups is not discussed at all.

Response:

The implications of the surface composition on hydrophobicity and adsorption are discussed in Lines 419-440.

419 **Hydrophobic interactions.** To test our hypothesis that the hydrophobic forces play an important role
420 in DNA adsorption at CM's, we measured the q_{eq} in mixtures of pure water and ethanol, and pure
421 water and isopropanol (Figure 4C). These alcohols have lower dielectric constant than water
422 ($\epsilon(\text{water})=80$, $\epsilon(\text{ethanol})=25$, $\epsilon(\text{isopropanol})=18$) so mixing them decreases the interfacial tension of
423 water in contact with a hydrophobic surface, decreasing the hydrophobic interactions.^{59,60} **At ~40%**
424 **of ethanol, the DNA conformation changes from a B-form predominant in aqueous solution to A-**
425 **form.⁶¹ The A-form is more compact than B-form and thus likely exhibits a higher charge density. If**
426 **the electrostatic interaction controls the adsorption of DNA on soot and charcoal, the transition in**
427 **conformation would suggest an increase in adsorption capacity as the alcohol concentration increases.**
428 **However, if** hydrophobic interactions influence adsorption, water-alcohol mixtures ought to retain
429 DNA in solution because the entropic drive for partitioning DNA from the solution to the hydrophobic
430 surface is diminished. We observed exactly that, a decrease in DNA adsorption when the volume
431 fraction of either ethanol or isopropanol in the solution increased (Fig. 4C). In addition, a q_{eq} in
432 isopropanol was consistently lower than in ethanol solution, as expected since isopropanol is less polar
433 than ethanol so there is a lower drive for DNA to escape it. An exception to this is a larger q_{eq} at 60
434 vol.% where we likely observed DNA precipitation in isopropanol but not in ethanol since higher ionic
435 strengths are needed for DNA precipitation in ethanol mixtures.^{62,61} Such adsorption behaviour was
436 also observed on graphene oxide,²⁹ which is significantly more hydrophilic than either soot or charcoal.
437 Since the bulk hydrophobicity of both CM's is similar, the higher q_{eq} at soot than charcoal in pure water
438 is perhaps a consequence of a strong heterogeneous distribution of hydrophobic sites at soot. This
439 heterogeneity at soot is likely reflected in a more complex modeling of DNA adsorption (eqs 5 and 6)
440 compared to charcoal (eq 4).

Comment:

• Line 218. What does an increase in surface charge density in divalent electrolyte mean? What would be the mechanism?

Response:

The increase in surface charge density is a consequence of divalent cation adsorption, as you speculate yourself in your next comment. We have clarified in the text:

232 **Surface properties.** In an inert electrolyte (100 mM NaNO₃), the PZC of soot (8.3 ± 0.1; Fig. S3a) and
233 charcoal (9.5 ± 0.1; Fig. S3b) was comparable to previous studies on CMs that used mass titration.^{41–44}
234 In CaCl₂ solutions, the PZC was lower than in NaNO₃ for both soot (7.7 ± 0.1; Fig. 1E) and charcoal (8.3
235 ± 0.2; Fig. 1F) likely reflecting an increase in surface charge density in divalent electrolyte solutions as
236 a result of cation adsorption. The IEP for both materials determined by electrokinetic measurements,

Comment:

• Line 221. The increase in IEP with increasing ionic strength may well be due to ion adsorption. This is not even mentioned.

Response:

Correct, cation adsorption causes the screening of negatively charged active sites. We do explain that in lines 238–240:

238 1G) while for charcoal it was ~ 3.0 in 1 mM CaCl₂ and 3.0 – 3.5 in 5 mM CaCl₂ (Fig. 1H). The increase of
239 IEP with an increase in ionic strength reflects a more efficient screening of negatively charged active
240 sites. IEP represents a pH value at which the electrokinetic potential equals zero, i.e. particle is not

Comment:

• Line 222. What are the so-called internal and external surfaces? How were they determined? The strong difference between IEP and PZC is really strange.

Response:

*A large difference in IEP and PZC as a consequence of different charging between internal and external surfaces is an established phenomenon for carbonaceous materials. We have already cited a paper that thoroughly discusses this phenomenon (43. Menéndez, J. A., Illán-Gómez, M. J., y León, C. A. L. & Radovic, L. R. On the difference between the isoelectric point and the point of zero charge of carbons. *Carbon* **33**, 1655–1657 (1995).) We have now offered some more explanations in the text:*

239 IEP with an increase in ionic strength reflects a more efficient screening of negatively charged active
240 sites. IEP represents a pH value at which the electrokinetic potential equals zero, i.e. particle is not
241 mobile under applied electric field, while PZC represents a pH value at which the net surface potential
242 of all particle surfaces equals zero. Since a higher PZC than IEP is lower than PZC, the surfaces that
243 control the particle mobility (external surfaces) are more negatively charged than particles whose
244 charge has little influence on mobility (internal surfaces) but can still be probed by proton adsorption,
245 i.e. the titration experiment.⁴³ indicates a—The difference between IEP and PZC implies a
246 heterogeneous distribution of surface charges where external particle surfaces are more negatively
247 charged than internal surfaces of both soot and charcoal;⁴³ and suggesting suggests that both soot
248 and charcoal that both behave as negatively charged surfaces in circumneutral solutions.

Comment:

- Line 248. What are inner particle surfaces??

Response:

Find our answer to your previous question. We have changed the wording here from “inner surface” to “internal surface” and “outer” to “external” to be consistent with our previous use of the term and the literature:

271 the adsorption would be minimal and the capacity would be close to zero. However, a significant
272 amount of DNA is still adsorbed: at both soot and charcoal there is still ~86% of DNA of the capacity
273 at pH=3. This cannot be due to adsorption at ~~inner-internal~~ particle surfaces that are more positive
274 than the ~~outer-external particle surfaces~~ (Figure 1E-F) because the ~~outer-external~~ surfaces are even
275 more negative at circumneutral pH (<-10 mV, Fig. 1G-H) thus repelling DNA. This suggest that the
276 electrostatics is not the only interaction governing the adsorption.

Comment:

- Line 254 and after. Binding through cation bridges is never considered whereas this could well occur.

Response:

*We have in fact considered “cation bridging” but we avoid using the term “cation bridge” because it is a generic vernacular and likely confusing for a wider chemistry community since **the ion bridge** is a term defined by IUPAC that means something completely different (a device that connects oxidation and reduction half cells in the electrochemical cell and maintains electrical neutrality). Instead, throughout the manuscript, we refer to “charge screening” or “the screening of electrostatic repulsion” to explain an increase in adsorption capacity of negatively charged DNA at a negatively charged surfaces as a function of increased ionic strength. This is a better term because “cation bridging”, in its strict sense, cannot explain such behaviour for monovalent cations.*

Comment:

- Line 275 and after. As said in the general remarks, this whole part is meaningless. For instance the transition from multilayer to monolayer deduced from the slightly best fit of Sipps equation does not make any sense.

Response:

See our answer to your “general remarks”.

Comment:

- Line 304 and after. Same remark as before. The fit to a model does not imply that the assumptions underlying the model are verified.

Response:

That statement is a generality and is correct for any form of data modelling. See our previous answers to understand our reasoning in using the combination of modelling and material characterisation to infer mechanism(s).

Comment:

- Line 319. Each DNA occupies three active sites?????

Response:

We have removed that sentence as it might mislead the readers.

340 depends only on the rate of DNA adsorption on active surface sites and not the rate of its transfer
341 through the solution to the particle. ~~Based on the assumptions of the Ritchie model,⁵¹ we deduce that~~
342 ~~the adsorption is dominated by the interaction with adsorption sites and not by the lateral interactions~~
343 ~~between neighbouring molecules and that each DNA molecule occupies three active sites ($n=3$).~~

Comment:

- Line 338 and after. Using a longer DNA could be an interesting idea. Still, no information about the various conformations of DNA is provided (in particular what role does ionic strength play on that conformation?). Furthermore, comparing particle size with DNA size and configuration could have provided relevant information.

Response:

We have added an explanation to the manuscript.

362 **Adsorption of long DNA.** In soils, the length of DNA influences the q_{eq} ^{52,53,53,54} and likely the overall
363 adsorption mechanism. To explore the role of DNA length on adsorption to CMs, we collected
364 adsorption isotherms using <2000 kb DNA (long DNA) in 100 mM NaCl and in pure water (Figure 4A).
365 ~~Because of charge screening of DNA within the ion atmosphere,^{54,55} the DNA in 100 mM NaCl is more~~
366 ~~coiled compared to DNA in water. Since supercoiled DNA adsorbs less to sand particles compared to~~
367 ~~linear or circular DNA,³ the change in conformation cannot alone explain higher q_{eq} in 100 mM NaCl~~
368 ~~compared to water.~~ Similarly to q_{eq} for ~30 kb DNA (short DNA) (Figure 2B-C), q_{eq} for long DNA at
369 charcoal is larger than at soot in 100 mM NaCl. However, this is not the case in pure water where q_{eq}
370 is higher at soot than at charcoal. This is the only instance where adsorption at soot was higher than
371 at charcoal (Fig. 2B-C, Table S3). These observations can be explained by enhanced hydrophobic
372 interactions in pure water compared to electrolytes where charges give rise to electrostatic attractive
373 interaction.

Comment:

- Line 389 and after. How does DNA behave in mixtures of water and alcohol.

Response:

The DNA form changes from a likely B-form to likely A-form as the ethanol concentration increases. Since the A-form is more compact than B-form, the conformation transition would suggest somewhat higher charge density of the DNA polymer and would imply larger adsorption capacity. However, we see exactly the opposite (a decrease in adsorption capacity with increased ethanol concentration) so the increase in the hydrophobicity is the only apparent explanation. We have added a few lines to address this issue.

419 **Hydrophobic interactions.** To test our hypothesis that the hydrophobic forces play an important role
420 in DNA adsorption at CM's, we measured the g_{eq} in mixtures of pure water and ethanol, and pure
421 water and isopropanol (Figure 4C). These alcohols have lower dielectric constant than water
422 ($\epsilon(\text{water})=80$, $\epsilon(\text{ethanol})=25$, $\epsilon(\text{isopropanol})=18$) so mixing them decreases the interfacial tension of
423 water in contact with a hydrophobic surface, decreasing the hydrophobic interactions.^{59,60} At ~40%
424 of ethanol, the DNA conformation changes from a B-form predominant in aqueous solution to A-
425 form.⁶¹ The A-form is more compact than B-form and thus likely exhibits a higher charge density. If
426 the electrostatic interaction controls the adsorption of DNA on soot and charcoal, the transition in
427 conformation would suggest an increase in adsorption capacity as the alcohol concentration increases.
428 However, if hydrophobic interactions influence adsorption, water-alcohol mixtures ought to retain
429 DNA in solution because the entropic drive for partitioning DNA from the solution to the hydrophobic

Review 2.

Comment:

1. This contribution by Jelavic et al. concerns the evaluation of the adsorption capacity of soot and charcoal surfaces with respect to DNA as a function of physicochemical medium composition, including change in pH, solution ionic strength, presence/absence of divalent cations, eDNA length, under both equilibrium and non-equilibrium conditions. In addition to classical batch adsorption experiments, authors resort to a suite of spectroscopic techniques (XPS, XRD, Raman, BET) to characterize the surface of their adsorbing materials and they analyse the obtained adsorption data with help of classical models e.g. Langmuir, Sips, Freundlich isotherms, etc.

Overall, I think this paper deserves publication pending, however, major revisions listed below.

1. The reader misses a general discussion on the very mechanisms driving the adsorption of DNA on their tested soot and charcoal surfaces, with full integration of a consistent and complete cross-examination of the data derived from the various techniques and models they adopt. In the current form, the manuscript lacks this overall discussion, and the latter would help to support the statement by the authors "this study provides a fundamental basis for dsDNA-CM interactions". As far as I can judge, most of the elements advanced by the authors to explain the adsorption patterns they conclude on (i.e. relative importance of electrostatics and hydrophobic interactions, heterogeneities of adsorbing surfaces, etc) are already mentioned in the introduction section (p.2) and are known from classical physicochemical literature on e.g. protein adsorption (see e.g. the work by Norde W. et al, etc). In turn, the authors should better clarify the new rationale they provide as compared to that from existing literature on a wider perspective (protein/macromolecules adsorption on heterogeneous surfaces).

Response:

Thank you for your suggestion. We have added sentences throughout the manuscript to emphasise the fundamental novelty of our work and a "bigger picture" implications for the environmental DNA research.

77 studies suggest that the ratio of hydrophilic and hydrophobic areas in carbonaceous materials
78 determines their overall interaction with dsDNA, with hydrophobic interactions becoming dominant
79 in materials rich in graphene-like surfaces. However, graphene-like materials are rare in the
80 environment and it is unclear to which extent our current understanding of DNA interactions with
81 carbonaceous materials is applicable to environmentally common surfaces such as soot and charcoal.

441 **CONCLUSION**

442 Elucidating the role of environmentally common CMs such as soot and charcoal in adsorption and
443 stabilization of eDNA is important for better understanding of its cycling in environment. This study
444 revealed showed that the adsorption capacity of dsDNA at soot and charcoal in general follows trends
445 observed at graphene and graphene oxide surfaces. The adsorption capacity of dsDNA increases as pH
446 decreases and as ionic strength increases, and it is generally higher for solutions containing divalent
447 compared to monovalent cations. Such behavior reveals that electrostatic interaction contributes to
448 DNA-CM binding since both soot and charcoal, and DNA are negatively charged at circumneutral pH
449 but become positive at lower pH. That the adsorption capacity is generally higher for solutions
450 containing divalent compared to monovalent cations suggests that attraction is, to an extent,
451 established by charge screening between negatively charged surfaces and DNA. As revealed by
452 adsorption modeling, the shape of adsorption isotherms in solutions of different pH and composition
453 was similar but different between short and long DNA suggesting that adsorption mainly depends on
454 the length of the DNA molecule but less so on the composition of the surface or the solution. However,
455 the distribution of hydrophobic areas on soot and charcoal surfaces determine the extent to which
456 the hydrophobic interactions will take place. Both soot and charcoal are similarly hydrophobic as
457 evidenced by their water adsorption behavior. However, the contribution of hydrophobic interaction
458 at soot was much stronger suggesting that regions which interact hydrophobically with DNA are more
459 suitably distributed to allow adsorption compared to the same regions at charcoal. The majority of
460 dsDNA adsorbs within minutes at both CMs with the activation energy of $\sim 50 \text{ kJmol}^{-1}$ suggesting a
461 strong, perhaps covalent binding. DNA that is bound so strongly to a surface likely cannot be desorbed
462 by common extraction techniques suggesting that a wealth of genomic and ecologic information might
463 remain hidden in samples after the extraction. Our results imply that dsDNA binds to both CM's by

Comment:

2. The reader is missing a quantitative assessment of the hydrophobicity level of the soot and charcoal surfaces (quite central in the manuscript), e.g. via macroscopic contact angle measurements or (better) via Atomic Force Microscopy (see e.g. Francius et al. ACS Applied Bio Materials 2021, 4, 2614-2627 or Nanoscale 2021, 13, 1257-1272). Related to this latter point, the reader is generally missing a physicochemical evaluation of the sorbing surface properties at the proper molecular scale as e.g. achieved with help of Atomic Force Microscopy operating in force spectroscopy mode with use of functionalized probes (e.g. decorated by -CH₃ or charged functional groups) or even a direct probing of the surface-DNA interaction upon functionalization of the AFM tip with tested DNA macromolecules (see e.g. Beaussart et al. Nanoscale 2018, 10, 3181 for the proof of principle). Such experiments would have the advantage to probe directly, at the molecular level, the operational interactions and the way these are impacted by surface heterogeneity (elaboration of spatially-resolved interaction maps) and possibly by changes in DNA conformation during the very interaction processes. These elements are missing from the literature quoted by the authors (see work on protein adsorption in literature).

Response:

Contact angle measurements on finely divided powder materials is burdened with many issues so we have instead decided to assess the hydrophobicity with water vapour adsorption which is more reliable than, e.g., Washburn method, and more routine and accessible than, e.g., heat of immersion measurements. Indeed, Chemical Force Mapping (CFM) would reveal the extent of surface heterogeneity with respect to hydrophobic and hydrophilic areas which would be an invaluable information to our

study. However, AFM on non-ideally-flat surfaces such as soot and charcoal particles, and in particular any kind of Force Spectroscopy is far from trivial and it is a study on its own. For the future, we are considering to chemically modify graphite to produce various surface oxygen groups with different spatial distribution and then assess heterogeneity with CFM to precisely infer its influence on DNA adsorption. However, at the moment, we believe that the assessment of hydrophobicity provided by water adsorption gives us the best we can do within this study and it gives us satisfactory answers.

Comment:

3. Authors should mention in the very body of the main text the Table summarizing the models they used to analyse their equilibrium and kinetic adsorption data. I doubt that the DNA-sorbing surface interactions are generally included in the classical models the authors refer to. Such model extension (which exist in literature) would help the reader to compare the pristine adsorption capacities of soot and charcoal and the relative contributions of the interactions (i.e. deconvolution between non-specific and specific (chemical) components of the adsorption isotherms and adsorption kinetics). For the sake of illustration, non-DLVO behavior is expected for surface-macromolecule interactions in the presence of multivalent ions and/or in concentrated electrolyte as the result of ion-ion correlations. Do this apply to the systems of interest in the manuscript ?

Response:

We agree that the isotherm models we use are not optimal for description of adsorption of DNA to a surface but we hold that they are still very useful for comparing adsorption at similar surfaces, under varying solution conditions. We do not use adsorption modelling to infer an adsorption mechanism of DNA at soot or charcoal, but to offer a quantitative description of different shapes of isotherms, and by combining these differences with the material properties obtained from other techniques described in the manuscript, to infer the adsorption mechanism. Instead of relocating the Table to the main text (which would likely place too much emphasis on it), we have added a few more sentences explaining our approach:

87 function of pH, ionic strength, solution composition, time and DNA length. We used isotherm
88 modelling to quantify differences in isotherm shapes. By evaluating how the surface properties of soot
89 and charcoal influence the adsorption of DNA as a function of solution composition, we infer a likely
90 adsorption mechanism. We propose that, besides electrostatic forces, hydrophobic interactions play

Comment:

4. The authors should discuss the relevance and applicability of their findings within a more general environmental context. More specifically, the authors focus here on well-defined DNA macromolecules (from salmon sperm, motivations of this choice ?) whereas eDNA involves macromolecules differing with respect to conformation,

chemical composition, origin, degradation, etc. This opens the debate on how the very heterogeneity of the macromolecules in terms of their defining physicochemical properties affects the adsorption capacities of soot and charcoal (themselves defined by physical and chemical heterogeneities). On the basis of their own data and of known sorption-mechanisms reported in literature (related to competitive adsorption features, etc), the authors could discuss, at least qualitatively, these various points.

Response:

We chose 30 bp salmon sperm DNA because it is relatively cheap and easy to work with, i.e., we needed large amounts of DNA for our experiments and we needed to reach initial DNA concentrations of app. 2 mgml⁻¹ to produce reliable and comprehensive isotherms. This would not be financially feasible with any other commercially available DNA, nor longer, home-extracted DNA. Since 30 bp is on the shorter end of what is routinely extracted, we compared its adsorption to 2000 bp salmon sperm DNA. We have expanded a bit about this in the manuscript:

149 **Batch adsorption experiments**

150 **Materials.** We used low molecular weight salmon sperm double stranded DNA (lyophilised powder,
151 Sigma Aldrich) with a size of ~30 base pairs (bp) because it is easily accessible in large amounts and
152 concentrations required for obtaining reliable adsorption isotherms. Since 30 bp is on the shorter end
153 of extracted environmental (ancient) DNA, ~~except for a set of experiments where~~ we looked into the
154 influence of DNA length on adsorption capacity of soot and charcoal ~~where we used salmon sperm by~~
155 ~~comparing it to adsorption of~~ double stranded DNA salmon sperm solution (UltraPure, 10 mgml⁻¹,
156 ThermoFischer Scientific) with the size of ≤2000 bp. We used DNA LoBind tubes (Eppendorf) and

441 **CONCLUSION**

442 Elucidating the role of environmentally common CMs such as soot and charcoal in adsorption and
443 stabilization of eDNA is important for better understanding of its cycling in environment. This study
444 revealed showed that the adsorption capacity of dsDNA at soot and charcoal in general follows trends
445 observed at graphene and graphene oxide surfaces. The adsorption capacity of dsDNA increases as pH
446 decreases and as ionic strength increases, and it is generally higher for solutions containing divalent
447 compared to monovalent cations. Such behavior reveals that electrostatic interaction contributes to
448 DNA-CM binding since both soot and charcoal, and DNA are negatively charged at circumneutral pH
449 but become positive at lower pH. That the adsorption capacity is generally higher for solutions
450 containing divalent compared to monovalent cations suggests that attraction is, to an extent,
451 established by charge screening between negatively charged surfaces and DNA. As revealed by
452 adsorption modeling, the shape of adsorption isotherms in solutions of different pH and composition
453 was similar but different between short and long DNA suggesting that adsorption mainly depends on
454 the length of the DNA molecule but less so on the composition of the surface or the solution. However,
455 the distribution of hydrophobic areas on soot and charcoal surfaces determine the extent to which
456 the hydrophobic interactions will take place. Both soot and charcoal are similarly hydrophobic as
457 evidenced by their water adsorption behavior. However, the contribution of hydrophobic interaction
458 at soot was much stronger suggesting that regions which interact hydrophobically with DNA are more
459 suitably distributed to allow adsorption compared to the same regions at charcoal. The majority of
460 dsDNA adsorbs within minutes at both CMs with the activation energy of ~50 kJmol⁻¹ suggesting a
461 strong, ~~perhaps covalent~~ binding. DNA that is bound so strongly to a surface likely cannot be desorbed
462 by common extraction techniques suggesting that a wealth of genomic and ecologic information might
463 remain hidden in samples after the extraction. Our results imply that dsDNA binds to both CM's by

Comment:

5. The authors refer to inner and outer particle surfaces, to shift of IEP and PZC with changing the concentration of 2:1 electrolyte. Authors should rather refer to inner and outer Helmholtz planes (accepted nomenclature in colloidal physical chemistry) and comment on the aforementioned shifts in relation to positioning of divalent cations at iHp or oHp and their extent of specific adsorption. Authors are referred to H. Lyklema, Fundamentals of Interface and Colloid Science, Vol. II, for that purpose. In addition, authors mention their measurement of zeta-potential. As a matter of fact, they do not explicitly state that they resort to (I think) DC electrophoresis measurements (measuring electrophoresis conditions should be specified, e.g. value of applied field ? etc), and they measure an electrophoretic mobility (not a zeta potential) that is subsequently converted into a "zeta-potential value". Authors should specify the nature of the equation used for the conversion (Smoluchowski, Debye-Onsager, etc ?) from mobility to zeta-potential and the extent of applicability of the adopted equation with regards to the very size and charge of the analyzed particles. It would have been further valuable to actually measure the dependence of the electrophoretic mobility of soot and charcoal on electrolyte concentration prior to AND after DNA adsorption. Upon application of soft particle and soft surface electrokinetic concepts (see e.g. Maurya et al. Journal of Colloid and Interface Science 2020, 558, 280 and Duval et al. Current Opinion in Colloid and Interface Science 2010, 15, 184-195), the authors could have then possibly probed the way surface electrostatics comes into play (or not) in controlling macromolecule adsorption process.

Response:

When we refer to inner and outer surfaces, we don't refer to Helmholtz planes but to the different surfaces on soot and charcoal particles, a phenomena described in the literature (e.g. Ref. 43 - Menéndez, J. A., Illán-Gómez, M. J., y León, C. A. L. & Radovic, L. R. On the difference between the isoelectric point and the point of zero charge of carbons. Carbon 33, 1655–1657 (1995).): a) within pores (inner) that do not influence the zeta potential value and b) on the particle surface that in fact influence the zeta potential. We have added a few words to clarify this:

232 **Surface properties.** In an inert electrolyte (100 mM NaNO₃), the PZC of soot (8.3 ± 0.1; Fig. S3a) and
233 charcoal (9.5 ± 0.1; Fig. S3b) was comparable to previous studies on CMs that used mass titration.^{41–44}
234 In CaCl₂ solutions, the PZC was lower than in NaNO₃ for both soot (7.7 ± 0.1; Fig. 1E) and charcoal (8.3
235 ± 0.2; Fig. 1F) likely reflecting an increase in surface charge density in divalent electrolyte solutions as
236 a result of cation adsorption. The IEP for both materials determined by electrokinetic measurements,
237 however, was significantly lower: for soot, IEP in 1 mM CaCl₂ was ~ 3.4 and in 5 mM CaCl₂ ~ 3.6 (Fig.
238 1G) while for charcoal it was ~ 3.0 in 1 mM CaCl₂ and 3.0 – 3.5 in 5 mM CaCl₂ (Fig. 1H). The increase of
239 IEP with an increase in ionic strength reflects a more efficient screening of negatively charged active
240 sites. IEP represents a pH value at which the electrokinetic potential equals zero, i.e. particle is not
241 mobile under applied electric field, while PZC represents a pH value at which the net surface potential
242 of all particle surfaces equals zero. Since A higher PZC than IEP is lower than PZC, the surfaces that
243 control the particle mobility (external surfaces) are more negatively charged than particles whose
244 charge has little influence on mobility (internal surfaces) but can still be probed by proton adsorption,
245 i.e. the titration experiment.⁴³ indicates a—The difference between IEP and PZC implies a
246 heterogeneous distribution of surface charges where external particle surfaces are more negatively
247 charged than internal surfaces of both soot and charcoal,⁴³ and suggesting suggests that both soot
248 and charcoal that both behave as negatively charged surfaces in circumneutral solutions.

Thank you for noticing the lack of method description. We have added some explanations:

134 For the electrokinetic measurements, we used a suspension of 1 mgml⁻¹ of soot and charcoal prepared
135 with 1 and 5 mM CaCl₂. We titrated a 10 ml suspension with 0.05 mM HCL in 0.5 µL steps and
136 simultaneously recorded pH and ζ potential using a Stabino instrument (Colloid Metrics GmbH,
137 Germany). The instrument contains a PTFE chamber with an oscillating piston that is slightly negatively
138 charged. A particle solution is added and van der Waal forces cause particle adsorption at the wall,
139 yet a fraction is immobilized. Due to the movement of the piston a mobile cloud of double layer is
140 formed and set in motion. Such oscillating ion cloud generates a voltage, which is captured by two
141 separate electrodes, defining the streaming potential of the solution, which is proportional to the zeta
142 potential of the particles. The Stabino streaming potential method can measure across a large particle
143 size range (0.3nm-300µm) and particle concentrations up to 40 vol.%. Moreover, optical properties of
144 the liquid are not relevant for its measurement, unlike electrophoresis method, which may be
145 challenging when working with soot and charcoal.

Regarding the measurement of electrophoretic mobility before and after the adsorption of DNA- our results demonstrate that the surfaces of soot and charcoal are highly heterogeneous with respect to surface charge and (likely) hydrophobicity. This heterogeneity would significantly limit and likely even prevent the applicability of any advanced surface adsorption model.